Aus dem Institut für Biologie der Humboldt-Universität zu Berlin

DISSERTATION

Reproductive strategies of K/T-crossing Theria: Neonate and postnatal development of the morphotype of Marsupialia and Placentalia (Mammalia)

Zur Erlangung des akademischen Grades
d o c t o r r e r u m n a t u r a l i u m (Dr. rer. nat.)
im Fach Biologie

eingereicht an der
Mathematisch-Naturwissenschaftlichen Fakultät I
der Humboldt Universität zu Berlin

Dipl. Biol. Kirsten Szdzuy
geboren am 29.10.1976 in Eberswalde-Finow

Präsident der Humboldt-Universität zu Berlin
Prof. Dr. Christoph Markschies

Dekan der Mathematisch-Naturwissenschaftlichen Fakultät I
Prof. Dr. Thomas Buckhout (PhD)

Gutachter:
1. Prof. Dr. Ulrich Zeller
2. Prof. Dr.Dr. Marilyn. B. Renfree
3. PD Dr. Barbara Tzschentke

eingereicht: 15. Februar 2006

Datum der Promotion: 06. Juni 2006

Table of contents

1 Introduction

“So in the general economy of any land, the more widely and perfectly the animals and plants are diversified for different habits of life, so will a greater number of individuals be capable of there supporting themselves. A set of animals, with their organisation but little diversified, could hardly compete with a set more perfectly diversified in structure, It may be doubted, for instance, whether the Australian marsupials, which are divided into groups differing but little from each other, and feebly representing, as Mr Waterhouse and others have remarked, our carnivorous, ruminant, and rodent mammals, could successfully compete with these well-pronounced orders. In Australian mammals we see the process of diversification in an early and incomplete stage of development.” (Darwin, 1859)

In his “Origin of Species”, Darwin (1859) tries to give an explanation for the divergent occurrence of marsupial and placental mammals today. Indeed, there are 4,350 placental (eutherian) species compared to only 260 marsupial species (Kemp, 2005). To explain these differences, several aspects, most associated with the different reproductive strategies of marsupials and placentals, must be discussed. One of the most obvious differences of marsupials and placentals, exist in the different developmental stage of their neonates. Therefore, this study focuses on the developmental degree and the adaptability of marsupial and placental neonates, in order to investigate one aspect which can have led to the divergent evolution of Marsupialia and Placentalia after the K/T-event, 65 million years ago.

1.1 Mammalian evolution

There are about 4,600 species of animals today that are called mammals because, despite an astonishing diversity of form and habitat, they all share a long list of characters not found in any other organisms, such as the presence of mammary glands, the single bone in the lower jaw, hairs and the neocortex of the forebrain. This makes them unambiguously distinct from their closest living relatives, and their unique characters together define a monophyletic taxon, the class Mammalia (Kemp, 2005).

Mammals clearly originated monophyletically from cynodont synapsids (Vaughan et al., 2000). The first mammals appeared in the Late Triassic period. Members of the family Morganucodontidae, from the late Triassic or earliest Jurassic period of Europe, represent the earliest known mammals (Mammaliaformes) (Carroll, 1993; Vaughan et al., 2000). These earliest mammals are similar in size and adaptations to living insectivorous mammals (Benton, 1991). But more important, there are some specific clues in the skeleton. There is a

complete secondary palate, a structure separating the nose from the mouth (Benton, 1991; Carroll, 1993; Maier, 1999). This shows that *Morganucodon* could breathe and feed at the same time, as can modern mammals, but not most modern reptiles. Taking in large amounts of food is a requirement for endothermy, where energy is being used at a fast rate to create heat. The advanced teeth (tribosphenic molar) allowed Morganucodon to process its food rapidly and efficiently, and it also had a complete covering of insulating hair (fur) (Benton, 1991). That refers to the acquisition of an endothermic metabolism (Hopson, 1973). The acquisition of endothermy enabled the first mammals to be active at night and thus to fill the niche of nocturnal insectivore, hitherto unoccupied by exothermic reptiles (Tyndale-Biscoe and Renfree, 1987). In the Mesozoic, mammals remained small animals with the largest being barely larger than a cat, and the vast majority of the size of living shrews, mice, and rats (Carroll, 1993; Kemp, 2005). For mammals, the Mesozoic, and especially the Cretaceous, was a time of experimentation. Behavioural, physiological, and anatomical changes that increased the efficiency of feeding, reproduction, and thermoregulation may have been critical. During the Mesozoic time, the basic mammalian structural plan was tested, retested, and perfected, and the major taxa were established (Vaughan et al. 2000). Modern mammals fall into three different, but closely related, groups: monotremes, marsupials, and placentals. These three are divided mainly by their methods of reproduction (Tyndale-Biscoe and Renfree, 1987; Zeller, 1999).

The order Monotremata of the subclass of the Mammalia known as the Prototheria developed in the early Cretaceous and consists today of only two families: Ornithorhynchidae (*Ornithorhynchus anatinus*) and Tachyglossidae (*Tachyglossus aculeatus*, *Zaglossus bruijnii*) (Griffiths, 1978). Morphologically, they closely resemble no other living mammals, and they possess several features frequently considered to be more typical of reptiles than of mammals. Monotremes lay eggs and incubate them in birdlike fashion, and yet they have hair and suckle their young (Vaughan et al., 2000). The relationship of monotremes to other mammals is unresolved (Archer et al., 1993). Morphological and palaeonotological evidence strongly suggests that monotremes diverged from an early mammalian ancestor before the divergence of marsupials and placentals (Vaughan et al., 2000; Kemp, 2005). Some molecular studies suggest that monotremes lie within the Theria and are most closely related to the marsupials (Penny and Hasegawa, 1997). Other molecular studies, however, indicate that the living monotremes are no more closely related to marsupials than to placentals and that they diverged from them about 163 to 186 million years ago (Messer et al., 1998). Today they are restricted in their distribution to Australia, New Guinea and Indonesian islands (Temple-Smith and Grant, 2001).

In contrast to the Monotremata, Marsupialia and Placentalia represent two viviparous evolutionary lines of the Mammalia that have been separated since the middle Cretaceous.

The earliest known stem placental *Eomaia*, and stem marsupial *Sinodelphys* occurred 125 million years ago in the part of Eurasia what is today China (Weil, 2002; Ji et al., 2002; Luo et al., 2003). Marsupialia and Placentalia form the monophyletic Theria, which are the sistergroup of the Monotremata (Novacek et al., 1988; Zeller, 1999; Zeller and Freyer, 2001). However, as a result of their long, independent history, marsupials differ structurally from placentals in many ways.

Marsupials differ from placentals mainly in their mode of reproduction. The young are born after a short gestation period at a very early stage of development. They are very small and are wholly dependent on the mother for a certain time after birth. During this long period of postnatal development each young is permanently attached to one teat. For these reason lactation is generally of long duration (Tyndale-Biscoe and Renfree, 1987; Tyndale-Biscoe, 2001; Zeller et al., 2001). The name “Marsupialia” is derived from the marsupium or pouch in which the young are carried (Vaughan et al., 2000). However, several kinds of marsupials do not develop a pouch (some didelphids, caenolestids, myrmecobids, and dasyurids). The earliest undoubted marsupial fossils are from the middle Cretaceous of China (Luo et al., 2003) and from the late Cretaceous of North America (Vaughan et al., 2000). Most of these fossils are jaws and teeth, and they can be identified as marsupial because they have three premolars and four molars, while placentals have four or five premolars and three molars (Benton, 1991). One of the most primitive marsupial families is the Didelphidae. Members of this family were present in North America during the late Cretaceous and have a nearly continuous fossil record there through the middle Miocene (Vaughan et al., 2000). Many experts believe that marsupials arose in North America and moved southward into South America in the Cretaceous and on to Australia, via Antarctica, in the late Cretaceous or earliest Tertiary (Woodburne and Case, 1996; Muizon et al., 1997; Vaughan et al., 2000). Today, only two important strongholds for marsupials remain: the Australian region (Australia, Tasmania, New Guinea, and nearby islands) and the Neotropics (southern Mexico, Central America, and South America) (Vaughan et al. 2000). Approximately 260 recent species dominate the mammalian fauna of Australia, and are part of the indigenous fauna of the Americas (Kemp, 2005). Where they have been isolated from placentals for long periods, marsupials have undergone remarkable radiation. Most marsupials have functional counterparts among placentals. Recent hypothesis of phylogenetic relationships among living marsupials have been given by Marshall et al. (1990), Baverstock et al. (1990), Szalay (1993), Luckett (1994), Springer et al. (1994, 1997), Horowitz and Sanchez- Villagra (2003), Westheide and Rieger (2004) and Asher et al. (2004). The several classifications of marsupials agree with one another as far as the recognition of seven major groups of living marsupials is concerned, although there is little agreement about their interrelationships to one another. From the seven marsupial groups are three American (Didelphoidea,

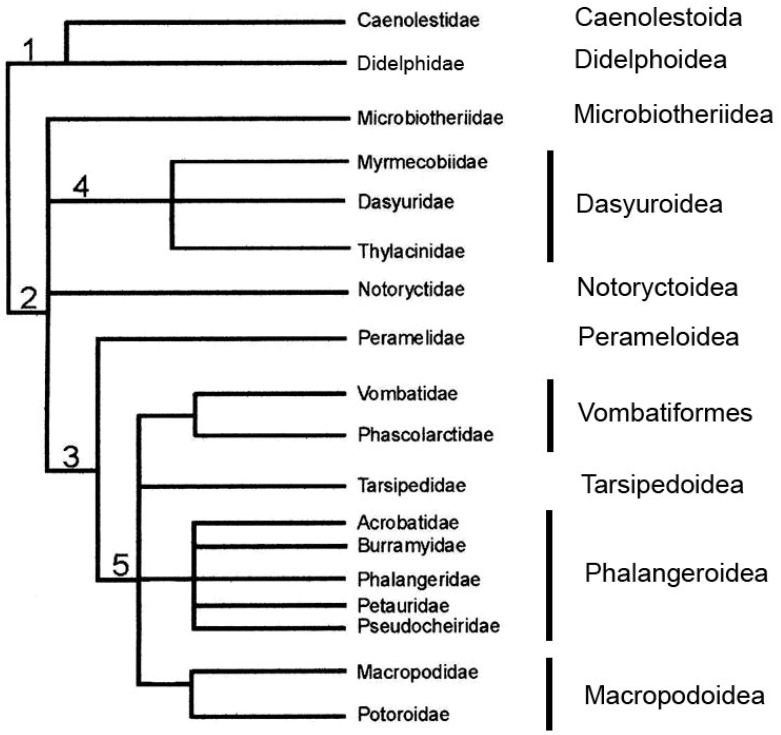

Fig. 1: Cladogram representing marsupial systematics as proposed by Luckett (1994). 1, Ameridelphia; 2, Australidelphia; 3, Syndactyla; 4, Dasyuroidea; 5, Diprotodontia. After Westheide and Rieger (2004).

Caenolestoida, Microbiotheriidea) and four Australian (Dasyuroidea, Notoryctoidea, Perameloidea, Diprotodontia) (Kemp, 2005). All studies confirm the monophyly of the Australidelphia including *Dromiciops* (Microbiotheriidea). In this study the cladogram by Luckett (1994) is favoured, because it is based on total evidence of molecular and morphological data (Fig. 1).

In contrast to Marsupialia, the Placentalia bear anatomically complete young after a relatively long gestation period (Vaughan et al., 2000; Zeller et al., 2001; Szdzuy et al., 2004). They retain their young inside until a late stage of development, nourishing them in the womb via the placenta. The placental young undergo a rapid postnatal development and achieve independency of their parents much earlier than marsupial young (Benton, 1999). The earliest fossil records of Placentalia are from the middle Cretaceous from China (Luo et al., 2003) and from Mongolia (Kielan-Jaworowska and Dashzeveg, 1989). Most of the fossils are fragments of jaws with partial tooth rows or isolated teeth, and they represent only parts of Asia, western North America, and a single locality in France (Vaughan et al., 2000). Late Cretaceous placentals probably played a variety of ecological roles. The diverse dentitions were seemingly adapted to insectivory, carnivory, and herbivory. The fossil record documents a late Cretaceous establishment of the evolutionary lines leading to the ungulates (Nessov and Kielan-Jaworowska, 1991; Archibald, 1996a) and a latest Cretaceous

establishment of the orders Insectivora (Eulipothyphla), Carnivora, and Primates. This fossil record also suggests a very early Cenozoic differentiation of the remaining extant orders of Placentalia (Vaughan et al., 2000). For long time studies based on the fossil record and morphological characters were accepted (Novacek and Wyss, 1986; Novacek, 1993). More recent molecular-based studies of several groups of workers using different methods and molecular databases have supported earlier divergence times of the modern lineages and describe also new relationships between the placental orders (Springer, 1997; Stanhope et al., 1998; Cao et al., 2000; van Dijk et al., 2001; Arnason et al., 2002; Springer et al., 2003). Springer et al. (2003) have conducted the most comprehensive analysis, using a large, diverse data set of nuclear and mitochondrial genes for 42 placentals representing all the living orders (Fig. 2). Their results generated divergence dates for the Afrotheria of about 102 million years ago. The two northern super-orders, Laurasiatheria and Euarchontoglires (composed of Euarchonta and Glires), separated about 94 million years ago. All the individual orders within these super-orders were differentiated from one another during the Late Cretaceous, between 82 and 77 million years ago, apart from the Afrotheria. In the Afrotheria, the Tenrecida separated from the Chrysochlorida about 65 million years ago; right on the Cretaceous-Tertiary (K/T)-boundary, and the three Paeungulata orders (sirenians, hyraxes, and elephants) diverged from one another about 60 million years ago. Springer et al. (2003) places most of the interordinal divergences in the Late Cretaceous, but most of the intraordinal splits at or after the end of the Cretaceous. Only Xenarthra, Eulipotyphla, Rodentia, and Primates have any intraordinal divergences between their constituent groups dated to the Late Cretaceous, these varying between 77 and 70 million years ago. Intraordinal divergences within Cetartiodactyla and Chiroptera commenced at 65 million years ago, the time of the K/T-boundary, and those within Carnivora, Perissodactyla, and Lagomorpha 10-15 million years after that event. After the K/T-boundary, there was an explosive appearance of new mammalian taxa right from the start of the Palaeocene (Kemp, 2005). Today, the Placentalia are by far the largest group of living mammals with about 4,350 species divided into usually eighteen recent orders and they are present or introduced to all continents of the world.

1.2 The K/T-mass extinction and its aftermath

The mass extinction at the end of the Cretaceous period, 65 million years ago, is known as the Cretaceous-Tertiary or K/T-event (Benton, 1991). It affected the world's biota to the extent that some 65-75% of the species disappeared in an instant (Kemp, 2005). On land, the main group to disappear were the dinosaurs. As the basic cause of the K/T-mass

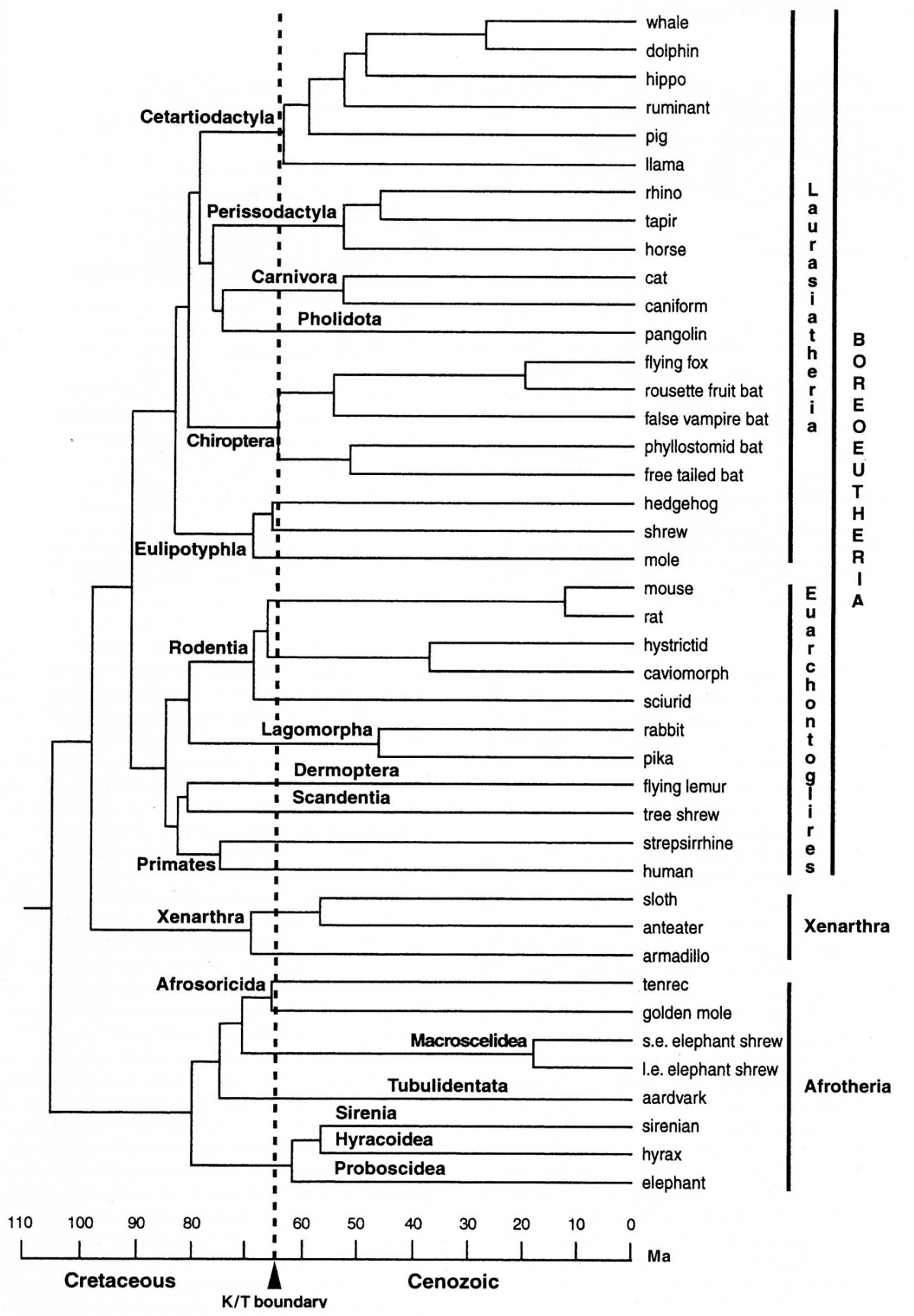

Fig. 2: Cladogram representing placental systematics as proposed by Springer et al. (2003). Molecular time scale for the placental orders. From Rose and Archibald (2005).

extinction it is generally accepted, that a large bolide, variously described as a meteor or a comet, struck the earth in the vicinity of Chicxulub, on the Yucatan Peninsula of southern Mexico (Keller et al., 2004). The world wide enriched iridium clay layer at precisely that time, the shocked quartz grains and microtectites, and the discovery of the submerged Chicxulub Crater all point in that direction (Kemp, 2005). There is also a sudden fall in the proportion of the stable isotope ^{13}C present in calcareous marine fossils at that time, which indicates a large fall in planktonic photosynthetic activity (Kemp, 2005). This would be compatible with a period of darkness following the impact. Also two studies of the megafloral fossil record across the K/T-boundary of Montana and on the Antarctic Peninsula indicate a radical climatic change at this time (Johnson and Hickey, 1990; Askin, 1992). Exactly at the K/T-boundary, many plants characteristic of warm conditions, such as palms, and araucariaceous conifers disappeared. There was a sudden and profound change in the flora and only 21% of the species survived into the Palaeozoic (Johnson and Hickey, 1990). Kemp (2005) summarises what is known of the environmental and ecological setting within which the mammals underwent their transition from the Mesozoic to the Tertiary. However it is, to say the least, sketchy and geographically very limited. In North America there was a gradual environmental shift during the late Cretaceous, involving an increase in temperature and humidity. At the K/T-boundary this process reversed, mean temperatures falling and conditions become dryer. Simultaneously, there was a dramatic shift in the vegetation, characterised by a reduction in angiosperms and an increase in ferns for a short time. One other important geographical feature at about this time was the regression of the sea level, causing a large reduction in coastal areas within North America, and also opening up a land connection across the Bering Strait to the Asian land mass (Smith et al., 1994).

The extinction of the dinosaurs at the end of the Cretaceous set the stage for the dramatic adaptive burst of mammals at the start of the Cenozoic. But there is a difference in the evolutionary development of marsupial and placental mammals after the K/T-event. Although sadly incomplete, the fossil record does suggest a competitive edge for placentals. After the mass extinction at the K/T-boundary, a massive diversification of placental mammals took place during the early Palaeocene and they developed rapidly in most parts of the world (Benton, 1991; Alroy, 1999). Within ten million years, nearly all the modern placental groups had appeared. However, it seems that the extinctions affected more marsupials than placentals (Archibald, 1982, 1991, 1996b). During the late Cretaceous, both groups seemed to be successful and they were reasonably diverse, although placentals were slightly more than marsupials. However, the marsupials lost two out of three families during the K/T-event, while the placentals seem to have lost none (Archibald, 1991, 1996b). Once the effects of the presumed end-Cretaceous bolide impact had waned, warm, geenhouse conditions pertained in the Eocene, with gentle climatic conditions (Prothero, 1997). Under these circumstances,

and in the absence of dinosaurs, a mammalian fauna that was essentially modern in ecological structure was rapidly established. Also the marsupials went through a radiation in South America from the Palaeocene onwards and in Australia in the Oligocene-Miocene (Kemp, 2005). However, they have not developed the systematic diversity of placentals. Only about 6 percent of the total numbers of species of living mammals are marsupials, but nearly 94 percent are placentals.

1.3 Reproductive strategies of Marsupialia and Placentalia

The recognition of a marsupial-placental dichotomy is based on a number of biological differences; primary among these are the contrasting reproductive patterns (Vaughan et al., 2000). Despite the fact that Marsupialia and Placentalia are both viviparous (as opposed to the oviparous monotremes), their respective modes of reproduction differ remarkably (e.g. Tyndale-Biscoe & Renfree 1987; Zeller 1999). Marsupials have a brief gestation period and bear almost embryonic young that have precocious forelimbs used to climb the mother´s hair to reach the nipple. They undergo most of their basic development during a long period of lactation while attached to the mother´s nipple and nourished by milk (Eisenberg, 1988; Tyndale-Biscoe and Janssens, 1988; Janssens et al., 1997). Placentals, in contrast, have long gestation periods and the young are much more developed at birth; they have a relatively short period of lactation (Eisenberg, 1988; Zeller et al., 2001). Placentalia can leave their offspring in a nest whereas the immature offspring of the marsupials is unable to live separate from their mother.

In the placental mammals, some species are born in a relatively naked, uncoordinated, and helples state and pass through a relatively long postnatal period of dependency on their parents. These are said to undergo altricial development (Eisenberg, 1988; Hill, 1992). Other species are more developmentally advanced when born and are said to show precocial development (Eisenberg, 1988). They are typically furred at birth or soon after; their eyes typically open soon; and they mature comparatively rapidly in locomotor ability and other respects, achieving independence of their parent's relatively early (Hill, 1992). Altricial and precocial developments are not monolithic categories; intergrades exist between the extremes, and a species may be relatively altricial in certain respects and relatively precocial in others (Kurta and Kunz, 1987).

Several aspects of reproduction reflect constraints imposed by small adult size. Small mammals share several features (Tab. 1). They are nocturnal, short-lived, and produce several litters in quick succession. The gestation period is short, the young at birth are often altricial, and are deposited in a nest (placentals) or attached to the teats in a brood area or

Tab. 1: A comparison of reproductive and life history characters in marsupial and placental mammals of small and large body size. From Tyndale-Biscoe and Renfree (1987).

	Small mammals		Large mammals	
	Placentalia	Marsupialia	Placentalia	Marsupialia
Activity	Nocturnal	Nocturnal	Diurnal	Nocturnal, crepiscular
Breeding	Polyoestrous	Polyoestrous	Polyoestrous/ monoestrous	Polyoestrous
Ovulation rate	Polyovular	Polyovular	Monovlar/ polyovular	Monovular
Gestation	Short to very short	Very short	Long	Short to very short
Development at birth	Altricial	Very altricial	Precocial	Very altricial
Thermoregu-lation at birth	Poikilothermic	Poikilothermic	Homeothermic	Poikilothermic
Jaw development at birth	Incomplete	Incomplete	Complete	Incomplete
Brain growth	Postnatal	Postnatal	Pre-natal	Postnatal
External protection	Nest, nidicolous	Pouch or brood patch then nest	No nest, nidifugous	Large pouch, no nest in majority
Lactation	Short, > gestation	Long, > gestation	Short, < gestation	Very long, 2 phases
Embryonic diapause	In some, lactational	In some, lactational and seasonal	In Carnivora, seasonal	In Macropodidae, lactational
Lifespan	1 year or less	1-2 years	More than 1 year	More than 1 year

pouch (marsupials). Most species of small marsupials lack a pouch and the young are deposited in a nest after a few weeks. In both groups brain growth, homeothermy, skull ossification and differentiation of the lower jaw and auditory ossicles occur after birth. As a consequence lactation in both groups is of longer duration than gestation (Tyndale-Biscoe and Renfree, 1987). It is reasonable to suppose that Mesozoic mammals, which were likewise small, displayed the above mentioned reproductive characteristics. Hill (1910), Müller (1969), and Lillegraven (1979) believed that the reproductive pattern of Mesozoic mammals of metatherian-eutherian grade would have been marsupial-like and that all reproductive apomorphies of living placentals could have evolved from such a condition. With the changing climates at the beginning of the Tertiary and the radiations of Angiosperms and Insecta, three major adaptive radiations of mammals occurred; placental herbivores and carnivores on the world continent of North America, Eurasia and Africa; placental herbivores and marsupial carnivores on South America, and the marsupial herbivores and carnivores on Australia-New Guinea (Kemp, 2005). An important aspect of all these radiations was increase in body size of species in most of the orders of Placentalia as well as in seven families of Marsupialia (Tyndale-Biscoe and Renfree, 1987). In recent years it has been

recognised that adult body size is a major factor in determining reproductive and life history strategies (Western, 1979; Bronson, 1985). Large size correlates with increased gestation length, reduced litter size and proportionally reduced maternal investment, slower growth rates, increased age at puberty, increased longevity and reproductive effort spread over several breeding seasons (Tab. 1).

Extension of the period of parental care must be accomplished by extension of intrauterine development (gestation) or extrauterine development (lactation) (Eisenberg, 1988). Amongst mammals, placentals have elaborated the first and marsupials the second alternative. The important apomorphic characters of the Placentalia are the lack of the shell membrane, early differentiation of the inner cell mass in most but not all species but in no marsupial, the precocious development of the chorioallantoic villous placenta in all species and the assumption of a variety of extrinsic controls of the corpora lutea in several orders (Tyndale-Biscoe and Renfree, 1987). All of these features are associated with the relatively longer retention of the embryo in the uterus and its delivery at a more advanced stage of development than in any marsupial. Conversely, the main apomorphic characters of marsupial reproduction are the fusion of the vaginae anterior to the ureters, the pseudo-vaginal canal, the choriovitelline placenta, the pouch and the special endocrine controls of early lactation and milk secretion (Renfree, 1983; Tyndale-Biscoe and Renfree, 1987). Most of these characters are associated with greater emphasis on lactation than gestation.

An overview of reproductive and developmental characters in the majority of mammalian species gives table III in the appendix.

1.4 Aims of the study

This study focuses on the reasons for the evolutionary differentiation between marsupial and placental mammals after the K/T-event. Especially the different reproductive strategies and the degree of development of neonates offer an interesting starting point for investigations. As previously mentioned, the most obvious difference between marsupial and placental mammals consists in the extreme immaturity of marsupial neonates and the advanced developmental degree in placental neonates. The neonate is obviously the most vulnerable stage in the mammalian life cycle (Hughes & Hall, 1988; Schultz et al., 2004) and therefore of special interest. The neonates of the Marsupialia and altricial Placentalia are small in size, naked, and completely dependent on maternal care. After Lillegraven (1979), these types of neonates are characteristic for the mammals at the K/T-boundary. With consideration of the climate cooling after the K/T-event, the high degree of immaturity in marsupial neonates could be a limiting factor. The small size of the marsupial neonate predisposes it to

hypothermia and potential desiccation. Although marsupials are identified mostly by the pouch, it seems likely that it is a relatively modern adaptation associated with the evolution of large size and the carriage of young for a long period (Russell, 1982). Due to the fact that marsupials at the K/T-boundary had probably no pouch, the young had to cope with the hazards of the environment.

The ability of the newborn animal to leave the environment in which it developed depends highly on the degree of maturation of the cardio vascular-respiratory system at the time of birth. At birth, the newborn's respiratory apparatus must be mature enough to take over the gas-exchange function previously provided by the placenta (Mortola, 2001). The lung of marsupials at birth is structurally immature yet functional as a gas exchanger (Baudinette et al., 1988). However, in placental mammals, the lung is obviously more developed at birth (Burri, 1974, 1985). Because the lung is one of the most important organs for the maintenance of life functions and also the prerequisite for the metabolic abilities, the lung development in marsupial and placental neonates will be investigated in this study.

Newborn mammals feature high energetic demands. Because of their smaller size and their greater propensity for heat loss, they should have higher weight-specific standard metabolic rates than adults (Mortola, 2001). Furthermore, newborns face the additional energetic cost of growth, development, and such maintenance functions as circulation, respiration, and digestation (Hill, 1992). For that purpose newborns should have high metabolic rates. Mammals have the ability to maintain a relatively constant body temperature (homeothermy) in spite of fluctuations in environmental temperature. To do this, they regulate the use of a metabolic by-product (heat). This energy demanding process is called endothermy. In the case of neonates, there are two possibilities. Either the young can carry out their own thermoregulation, in which case heat production to keep them warm will draw on their own chemical-energy resources. Or the parent(s) can keep the young warm (through heat flow from the parent(s) to the young), in which case the heat production to warm the young will draw directly on parental chemical-energy resources (Hill, 1992). However, each individual mammal must develop the ability to regulate its own body temperature by the time it achieves independent existence (Hulbert, 1988). With consideration of the climate cooling after the K/T-event in particular the metabolic abilities, important for maintenance of body functions, development, and thermoregulation, of marsupial and placental neonates will be investigated in this study.

This dissertation is part of a holistic view at the reproductive strategies of Marsupialia and Placentalia at the K/T-boundary. Two further studies attended to the morphotypes of the marsupial placenta (C. Freyer, completed) and the Eulipothyphla placenta (S. Siniza, not finished yet). In addition, my project will reconstruct the morphotypes of the marsupial and placental neonates respectively. For that purpose the investigations focus on the general

developmental degree, the lung development and the metabolic abilities of the neonates and the postnatal development of these characters. For the morphotype reconstruction two marsupial and four (five for the lung development) placental species are chosen. The Marsupialia are represented by *Monodelphis domestica* (Ameridelphia) and *Macropus eugenii* (Australidelphia). For the Placentalia three altricial born species and one (two for the lung development) precocial species of different placental orders are investigated. The first three are *Mesocricetus auratus* (Rodentia), *Suncus murinus* (Eulipothyphla), and *Tupaia belangeri* (Scandentia). The precocial born Placentalia are *Cavia aperea* (Rodentia) and *Macroscelides proboscideus* (Macroscelidea; only lung development). This selection should represent the variety of marsupial and placental mammals. For the morphotype reconstruction also data from the literature will be supplemented.

The aims of this holistic study can be summarised as follows:

1. Investigation of the general postnatal development in marsupials and placentals.
2. Histological and ultrastructural (SEM, TEM) investigation of the neonatal lung structure and of the postnatal lung development in marsupials and placentals.
3. Indirect calorimetry to determine the metabolic rate in neonates and during the postnatal period in marsupials and placentals.
4. Morphotype reconstruction of marsupial and placental neonates.

The neonatal lung structure and postnatal lung maturation of marsupials were investigated before in several studies (Bremer, 1904; Krause and Leeson, 1975; Walker and Gemmell, 1983; Gemmell and Selwood, 1994; Cehun, 1994; Runciman, 1994; Runciman et al., 1996; Schmidt, 1996). Also the lung development of altricial and precocial born placentals was subject of investigations (Boyden and Tompsett, 1961; Burri, 1974, 1985; Thurlbeck, 1975; Brody and Vaccaro, 1979; Ten Have-Opbroek, 1980; Weibel, 1980; Lechner and Banchero, 1982; Winkler and Cheville, 1984; Castleman and Lay, 1990). Several studies were conducted to the metabolic development of marsupials and placentals (marsupials: Shield, 1966; Setchell, 1970; Baudinette et al., 1988; Singer, 1998; Frappell and Mortola, 2000; placentals: Dryden et al., 1974; Noblet and Dividich, 1981; Mortola, 1991; Bagatto et al., 2000). However, most of these studies focus on only one mammalian species and one topic, respectively. They all lack a comparative view at the lung structure and the metabolic abilities of newborn mammals. The here presented integrated study is the first, which combines lung structural and metabolic aspects and additionally includes six mammalian species in order to reconstruct the morphotypes of marsupials and placental neonates.

2 Material and Methods

2.1 Species examined

In the presented study six mammalian species were compared (Fig. 3). The aim of the survey was a comparison and a morphotype reconstruction of newborn Marsupialia and Placentalia. For the exemplary coverage of the Marsupialia the gray short-tailed opossum *Monodelphis domestica* (Ameridelphia: Didelphoidea, Fig. 3a) and the tammar wallaby

Fig. 3.1: The six species examined. *Monodelphis domestica* (a), *Macropus eugenii* (b), *Tupaia belangeri* (c), *Suncus murinus* (d), *Mesocricetus auratus* (e), and *Cavia aperea* (f). Photographs from the animal facility of the Institute of Systematic Zoology, except b (research colony of the University of Melbourne) and c (London Zoo).

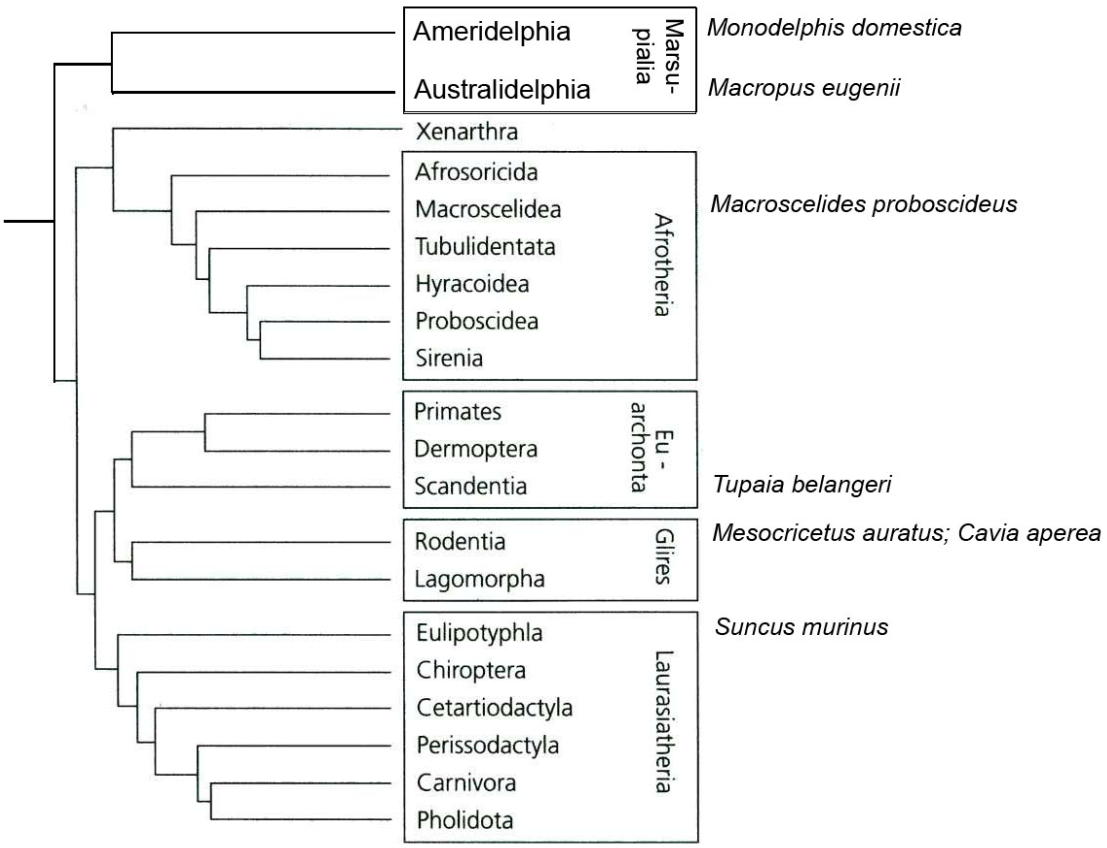

Fig. 3.2: The position of the species selected within the phylogenetic tree of the Mammalia, representing the main mammalian superorders. Cladogramm from Westheide and Rieger (2004).

Macropus eugenii (Australidelphia: Macropodoidea, Fig. 3.1 b) were examined. As representatives for the Placentalia the Belanger's tree shrew *Tupaia belangeri* (Euarchonta: Scandentia, Fig. 3.1 c), the musk shrew *Suncus murinus* (Laurasiatheria: Eulipothyphla, Fig. 3.1 d), the golden hamster *Mesocricetus auratus* (Glires: Rodentia, Fig. 3.1 e) and the guinea pig *Cavia aperea* (Glires: Rodentia, Fig. 3.1 f) were chosen. For the investigation of the lung structure, the short-eared elephant shrew *Macroscelides proboscideus* (Afrotheria: Macroscelidea) was supplemented additionally. This selection of marsupial and placental taxa was choosen to cover the main mammalian superorders in order to obtain a meaningful reconstruction of the marsupial and placental morphotype (Fig. 3.2).

Monodelphis domestica is bread regulary at the animal facility of the Institute of Systematic Zoology (Director: Prof. Zeller) at the Museum of Natural History in Berlin and was available for this project. For the dissertation breeding colonies of *Suncus murinus, Mesocricetus auratus* and *Cavia aperea* were established. The animals for the respective breeding groups were delivered from the Tierpark Berlin-Friedrichsfelde (*Suncus murinus, Cavia aperea*; Dr. Rudloff) and from the Institute of Zoology of the University of Halle (*Mesocricetus auratus;* Prof. Gattermann). For the examination of *Tupaia belangeri* pregnant females were borrowed from the German Primate Center Göttingen (DPZ, Prof. Fuchs, and Dr. Schmelting). The

offspring of these tupaias was used for the metabolic and lung structural investigations. The keeping and breeding of the five mammalian species took place with permission of the ethics commission (registration number ZH 104). The tammar wallaby was examined during a research stay at the Department of Zoology (Prof. Renfree) at the University of Melbourne, Australia. For the metabolic and histological studies several postnatal stages of pouch young were available from the research colony and collection of Prof. Renfree.

2.1.1 Monodelphis domestica

The gray short-tailed opossum *Monodelphis domestica* [Wagner, 1842] belongs to the family Didelphidae (Didelphoidea: Marsupialia), the South American opossums, part of the Ameridelphia. The didelphids are of special interest because they are the most ancient marsupial family, and may have been the stem from which all other New World and Australian marsupials were derived (Fadem et al., 1982; Zeller and Freyer, 2001). The natural distribution of *Monodelphis domestica* is from eastern Panama to central Argentina (Fadem et al., 1982). *Monodelphis domestica* is nocturnal and lives terrestrial or semiarboreal, reportedly living near water and building nests among rocks and in hollow logs (Fadem et al., 1982). The nutrition of Monode*lphis domestica* is omnivorous; they consume insects and other invertebrates, small vertebrates and various fruits (VandeBerg, 1999). The short tail, typical for the genus, is slightly prehensile and is used for carrying nesting material and for support during climbing. Head and body length of adult *Monodelphis domestica* ranges from 100-150 mm, with an additional tail length of 60–80 mm. Adult males weigh 90–155 g and females 80–100 g (Fadem et al., 1982). The coat colour is greyish-brown, with lighter underparts. *Monodelphis domestica* breeds throughout the year. The oestrus is induced by the presence of a strange male (Vandeberg, 1999). After a gestation period of 14 days they produce litters of 8-14 young with interbirth intervals of 4 ½ months (Tyndale-Biscoe and Renfree, 1987). Due to the fact that *Monodelphis* is a pouchless genus, the young are fixed firmly to the teat for the first 14 days; afterwards the female leaves her offspring in a nest. In danger the juveniles often ride on the females's back, clinging to her fur (Fadem et al., 1982).

2.1.2 Macropus eugenii

The tammar wallaby *Macropus eugenii* [Desmarest, 1817] belongs to the family Macropodidae (Macropodoidea: Marsupialia), which contains 67 species (MacDonald, 2002). Beside the Dasyuridae (70 species) they represent the largest group of Australian marsupials (Australidelphia). The family Macropodidae appears first in the late Oligocene or early Miocene of Australia (Vaughan et al., 2000). Although phylogenetically relatively young, the

tammar wallaby was included in the study as typical representative of the Australidelphia. Once widely distributed, *Macropus eugenii* is now limited to five islands off the coast of Western Australia and to Kangaroo Island in South Australia. The tammar wallaby inhabits areas such as coastal scrub, heath and dry sclerophyll forest, where dense low vegetation is available for daytime shelter and open grassy areas are available for feeding (Mate et al., 1999). The nutrition of the tammar wallaby is solely herbivorous and is based on native grasses. *Macropus eugenii* has long soft fur, dark grey-brown flecked with reddish-brown patches on the flanks and limbs, paler below. Tammar wallabies are sexually dimorphic, with females weighing 4-6 kg and males weighing 6-10 kg (Mate et al., 1999). The head and body length is 520-680 mm, with an additional tail length of 330-450 mm (Cronin, 2000). The tammar wallaby is a polyoestrous, seasonal breeder (January to June) which ovulates a single egg in an oestrous cycle of 28-29 days (26.5 days from diapause to birth) (Tyndale-Biscoe and Renfree, 1987; Mate et al., 1999). A single young is born after a gestation period of 29 days and remains in the pouch during the first 105 days, fixed firmly to one of the four teats of the female. With 250 days the young leaves the pouch permanently and with 270 days the lactation period ends (Tyndale-Biscoe and Renfree, 1987). A distinctive feature of the Macropodidae is the embryonic diapause. The female tammar wallabies give birth, mate one our later (Rudd, 1994) and ovulate about 40 hours later (Renfree and Lewis, 1996). They mate with multiple males. The new conceptus develops only into a blastocyst and then enters diapause, which persists usually until the following summer solstice. The hormonal signal that blocks further development of the blastocyst is produced in reponse to the sucking stimulus from the young in the pouch. When this impulse is missing, for example if the young is lost from the pouch, the quiescent blastocyst resumes development (Shaw and Renfree, 2001).

2.1.3 Mesocricetus auratus

As representative of altricially born Placentalia, the golden hamster M*esocricetus auratus* [Waterhouse, 1839] was examined. *Mesocricetus auratus* belongs to the family Muridae (Rodentia: Placentalia) in the suborder Sciurognathi and is a representative of the Rodentia, the largest mammalian order. Approximately 43 percent of all mammals are rodents, with 29 living families, 443 genera, and roughly 2004 species (Wilson and Reeder, 1993). Rodents are a phylogenetic old group, with origins supposed in the Mesozoic (Novacek, 1993). The earliest fossils are dating back to the late Paleocene of Asia and North America. Recently, the distribution of the golden hamster is restricted only to a small area of northwestern Syria (MacDonald, 2002). Hamsters are terrestrial and good diggers. Their natural habitats are dry, rocky plains or lightly vegetated slopes. They build nests which they construct within self-dug burrows (Whittaker, 1999). Hamsters are mainly herbivorous; primarily grain eaters, but they

also eat green plant shoots and roots, insects and fruits (Whittaker, 1999). They have small, compact, rounded bodies with short legs, short tails, large ears, prominent dark eyes, long whiskers, and sharp claws. The thick fur is brownish-orange at the back with a white underpart. Special features are the cheek pouches that consist of loose folds of skin. When hamsters forage they can push food into the pouches, which then expand, enabling them to carry large quantities of provisions to their underground storage chambers. They reach a head body length of 130 mm and a body weight of 80–100 g (Eisenberg, 1983). *Mesocricetus auratus* is reproductive active all the year round. Females are polyoestrous with a regular oestrous cycle lasting four days (Whittaker, 1999). After a gestation length of 16 days on average six to eight naked, blind young are born, which will be nursed for 15-21 days (Puschmann, 2004).

2.1.4 Suncus murinus

As another altricial Placentalia the musk shrew *Suncus murinus* [Linnaeus, 1766] was included in this study. *Suncus murinus* belongs to the family Soricidae (Eulipothyphla: Placentalia) in the former order Insectivora, which included hedgehogs, moles, tenrecs, golden moles and solenodons (Vaughan et al., 2000). However, recent DNA analyses suggest that the golden moles and tenrecs should be assigned to a new order, the Afrosoricida, which is part of the supraordinal assemblage that makes up the Afrotheria (Stanhope et al., 1998; Hedges 2001; van Dijk et al., 2001; Springer et al., 2003). Correspondingly, the shrews, moles, hedgehogs and solenodons became a new order, the Eulipotyphla (Murphy, 2001; MacDonald, 2002). The fossil record of Eulipotyphla may begin with the poorly known Batodon in the late Cretaceous of North America (Vaughan et al., 2000). An insectivorous lifestyle was common to many cretaceous eutherians. Due to the phylogenetic age of the group and the original insectivorous lifestyle *Suncus murinus* is an integral part in this study. *Suncus murinus* inhabits tropical and subtropical regions throughout Asia (Chang et al., 1999). Obviously they have a wide distribution. Bedford et al. (1997) mentioned Nepal, India, Japan and Indonesia as countries of origin. They inhabit wood, scrub and savannah. Shrews are very active and have a high metabolic rate, which demands feeding all the time. They are crepuscular and nocturnal and the natural diet consists of invertebrates, especially insects and their larvae, small vertebrates, but also herbivorous food (seeds, nuts, fruits) (Puschmann, 2004). The appearance of *Suncus murinus* is mouse-like but with a characteristically long, pointed nose and elongated skull. The eyes are small and the tail long and sparely haired. The fur is short, dense, velvety and grey coloured. A special feature of musk shrews are the musk glands at each side of the body. The head and body length is 80-100 mm. Body size and body mass vary regional. So female *Suncus murinus* from Nepal have a mean body mass of 50.8 g, whereas females

from Japan and Indonesia weigh 37.6 g on average (Bedford et al., 1997). In the tropics *Suncus murinus* is reproductive active all the year round and in temperate zones three to four litters per year can be expected (Puschmann, 2004). The ovulation is induced by mating (Bedford et al., 1997). After a gestation period of 31 days one to three naked and blind young are born. At the age of 17-20 days they will be weaned (Dryden, 1968).

2.1.5 Tupaia belangeri

The last altricially born Placentalia examined is the Belanger's tree shrew *Tupaia belangeri* [Wagner, 1841]. *Tupaia belangeri* belongs to the family Tupaiidae (Scandentia: Placentalia). This family consists of five genera and 19 extant species (Vaughan et al., 2000). Although the first fossil tupaiids appeared first in the middle Eocene, the origin of this group is assumed to be in the Mesozoic (Novacek, 1993). At their discovery at the end of the 18th century they were classified as sciurids at first. Gray (1825) rearranged the tree shrews to the Insectivora. Similarities to the Primates were realised first at the beginning of the 20th century. This resulted in a classification of the Tupaiidae into the Prosimia (Hertenstein et al., 1987). As a result of the uncertainties in the phylogenetic relationships, most modern systematists place them in an own order Scandentia (Butler, 1972; Kuhn and Schwaier, 1973; Luckett, 1980; Zeller, 1983, 1986 a, b; Yates, 1984). The natural habitats of the tree shrews are the tropical rainforests from South East Asia. The nutrition of the diurnal and semi-arboreal animals is omnivorous and contains fruits, insects and small vertebrates (Hertenstein et al., 1987). The appearance is similar to a squirrel, lank and of the size of a rat. Head and snout are acuminate. They have big eyes, the ears are naked and the long tail is usually heavily furred. The dense fur is brown or reddish coloured. The head and body length of *Tupaia belangeri* is 120-210 mm, with an additional tail length of 140-200 mm. The body weight is 200-270 g (Fuchs, 1999; Puschmann, 2004). Several authors suggested an 8-12 day (anovulatory) oestrous cycle in Tupaia, and ovulation is supposed to be induced by copulation (Fuchs, 1999). After a gestation period of 42-45 days tree shrews give birth to usually one to four (mostly two) naked and poorly developed young. Immediately after birth, the young are first nursed and then left by the female (Puschmann, 2004). For the first month of life, the young stay in a nest apart from the female, who visits them every 48 hours to nurse them (Fuchs, 1999; Puschmann, 2004). The lactation period ends with 38 days and with three months the offspring is full-grown and sexually mature.

2.1.6 Cavia aperea

To take into consideration the wide spectrum in the reproduction of the placental mammals, the precocially born Brazilian guinea pig Cavia aperea [Huckinghaus, 1961] was included in

the investigation. *Cavia aperea* belongs to the family Caviidae (Rodentia: Placentalia) in the suborder Hystricognathi and is part of the very varied order of Rodentia. The natural distribution area of Cavia aperea is in South America in Peru, Argentina, Paraguay and in eastern and southern Brazil (North, 1999). Guinea pigs are cursorial rodents which do not burrow, but may take advantage of burrows and scrapes dug by other animals (North, 1999; Puschmann, 2004). Their lifestyle is diurnal (Puschmann, 2004), but is described also as crepuscular and nocturnal (North, 1999). The preferred habitats are open grasslands of the flat and hill country, covered with scrubs, they are rarely seen in moutains up to 4200 m. Guinea pigs are sociable animals and live in small groups. They are philopatric in territories with steady tracks (Puschmann, 2004). The nutrition is exclusively herbivorous and consists of grasses, herbs and leaves. The body of *Cavia aperea* is short and robust and the head large. The eyes are fairly large and alert, the ears big but close to the head and there is no tail present. The grey-brown fur is coarse and easily shed when the animal is handled. The head body length of adult animals is up to 200 mm, the weight is between 450-700 g (Trillmich, 2000). The females are polyoestrous all the year round. The length of the oestrous cycle is about 20 days. The duration of the oestrous is less than 12 hours and an early postpartum oestrus exists (at the day of birth; Puschmann, 2004). After a gestation length of 61 days one to five (mean 2.3) fully furred, cursorial active young with open eyes are born (Trillmich, 2000). Although the young are nursed for 15 days (Trillmich, 2000), they already take solid food on the day of birth or one to two days later (Puschmann, 2004).

2.2 Lung structure

2.2.1 Material / Stages

For the investigation of the lung structure of *Monodelphis domestica, Macropus eugenii, Mesocricetus auratus, Suncus murinus, Tupaia belangeri* and *Cavia aperea* histological series as well as electron microscopic preparations were produced. In all six species series of transverse sections of the whole body or the lung were cut from available collection material or fresh preserved stages. Outgoing from the neonatal stage, different postnatal ontogenetic stages, dependent on the respective developing speed of the species, were examined. In Mesocricetus auratus and Suncus murinus neonates and stages at the age of 4, 7, 11 and 14 days (in *Mesocricetus* additionally day 2) as well as adult stages were chosen. In *Tupaia belangeri* ontogenetic stages of neonates, 4 and 7 days old young were examined. Due to the fact that the precocial *Cavia aperea* is born already with an almost mature lung, only neonates, 4 days old young and for comparison an adult animal were investigated. In the case of *Monodelphis domestica* material from the collection of Prof. Zeller

was available. So comparable age stages, like neonate, 5, 8, 12, 14 and 21 days postnatal (dpn) were examined. Due to the relatively slow postnatal development in Monodelphis domestica additional ontogenetic stages of 4, 6 and 8 weeks as well as three months (adult) were investigated. All killings and preparations of the above mentioned species were carried out with permission of the ethics commission (registration numbers: *M. domestica, S. murinus, M. auratus* T 0072/03; *T. belangeri* T 0260/04; *C. aperea* T 0117/04). For the investigation of Macropus eugenii the lungs of 13 ontogenetic stages at the age of 1 to 142 days from the collection material of Prof. Renfree (Department of Zoology, University of Melbourne, Australia) were dissected, embedded in paraffin and transferred to Germany. The series of sections were cut in Berlin to guarantee a comparable treatment of the material. In comparison to Cavia aperea a neonatal and adult short-eared elephant shrew *Macroscelides proboscideus* (Macroscelidea: Placentalia) was included in the investigation of the lung structure of precocial placentals.

Theria can be confronted with the subclass Prototheria. To decide which developmental stage of lung development is original, a comparison with the phylogenetically old Monotremata makes sense. From the Hill Collection two nest- respectively pouch-young Monotremata (Ornithorhynchus anatinus, Tachyglossus aculeatus) were available. The torsos of both species were histologically examined for comparison. All taxa and ontogenetic stages examined as well as the information to the respective series of sections and electron microscopic preparations are summarized in table I (see appendix).

2.2.2 Methods: Light microscopy

For histological examination of the lung in neonates and small ontogenetic stages the whole torso, in larger stages only the lung, was fixed and embedded. All ontogenetic stages of *Monodelphis domestica, Mesocricetus auratus, Suncus murinus, Tupaia belangeri* and *Cavia aperea* were sacrificed with permission of the responsible ethics commission. To obtain an optimal fixation result the lungs were fixed by means of perfusion short after the animals were sacrificed with chloroform. The perfusion was carried out by insertion of a gauge needle into the superior vena cava via the infundibulum of the right ventricle of the pulmonary circulation. It was rinsed with Ringer's solution (physiological common salt solution) and fixed with Bouin's blend (15 parts picric acid, 5 parts formalin, 1 piece glacial acetic acid; Romeis, 1989). In addition the fixing agent was endotracheally instilled in the pulmonary interior. After one day fixing in Bouin the fixative was rinsed with 70 % ethanol. Torsos which contain bones were decalcified four days with a stock solution (100 ml dimethylsulphoxide, 15 ml nitric acid (65 %)), rinsed after that in sodium sulphate solution (5 %) and distilled water and transfered in 70 % ethanol. For the embedding of the material in paraffin an embedding

automat (Shandon Hypercenter XP) was used. During this process an ascending alcohol series (80 - 100 %) was passed and the preparations transferred afterwards via chloroform into fluid paraffin. After a day at 60°C in the warming box, the objects were imbedded in a paraffin block, and prepared for cutting. The transversal dissection in A and B series occurred in 7 to 12 µm thick cuts at a Leica SM 2000 R microtome. The sections were fixed with protein glycerol on slides, stretched at 40 °C on the heat plate and dried at 40°C in the warming box overnight. As preparation for the staining of the paraffin sections, it was necessary to draw the slides through xylene and one descending alcohol series (100 - 70 %) for deceration. The stainings of the A series were carried out according to the methods mentioned in Romeis (1989). Used stainings were Azan (Heidenhain, 1915), Trichrome (according to Masson, Goldner, 1938) or Haematoxylin (Harris, 1898), that was counterstained with a 0.1 % watery Eosin solution. The covering of the stained slices occurred either by hand or with a robotic coverslipper (Leica CV 5030) using the glue Pertex to cover the slides. The series were viewed at a Leica MZ 12 stereomicroscope in transmitted light. The drawings were created at the Zeiss Axioskop using a camera lucida. For important developmental stages a graphic representation of the bronchial tree was carried out. For this purpose each fifth section of a series was projected onto a sheet of graph paper. The medial and lateral boundaries of the lumens of large bronchi were plotted orientated at the axis. The points were linked with each other and drawn in a three-dimensional way onto a white sheet of paper. Digital photographs were taken at a Zeiss Axioskop 2 with a Prog/Res/3012 camera (Kontron Electronics) and post-edited at the computer (Adobe Photoshop 5.0).

2.2.3 Methods: Transmission electron microscopy (TEM)

The transmission electron microscopy works due to the long focal lengths with depths of focus which outdo that of the optical microscope by 300-1000 times (Bucher and Wartenberg, 1989). This insight into the ultrastructure of the tissue is an important complement to the light-microscopic findings. The special features of the electron microscope are the use of electrons instead of photons as "light beams" and the electro-magnetic lentils (Plattner and Hentschel, 1997). The TEM consists of a beam producing system (cathode, anode), a double condenser for the effective focusing of the electron beam, an aperture diaphragm for the reduction of the beam cross-section and a deflection system for the beam alignment and for the equal illumination of the preparation. The image producing electrons have to pass the contrast aperture and the enlarging lentils of objectives and projectives in order to reproduce the picture on the fluorescent screen (Plattner and Hentschel, 1997). A prerequisite for the investigation with the TEM is that the sections have to be very thin (electrons can penetrate only thin layer without disturbance) and dry (in the

vaccum not dried preparations would begin to boil). In order to meet these conditions, the cell structure must be chemically stabilised sufficiently, that means fixed, before the cells can be dried, impregnated and hardened for the manufacturing of ultrathin sections with epoxies or methyl acrylates. For an optimal fixing-result the lung was rinsed (Ringer's solution) and fixed by means of perfusion (see light microscopic methods). After fixation smaller pieces of pulmonary tissue were dissected. The preparations were fixed, according to Sabatini et al. (1963), with a glutaraldehyde blend (6 ml glutaraldehyde, 6 ml paraformaldehyde, 45 ml 0.2 molar cacodylate buffer) and postfixed with osmium tetraoxide (double fixing). Glutaraldehyde stabilises particularly the proteins and osmium tetraoxide stabilises particularly the lipids. After a fixing time of two hours in the glutaraldehyde blend, the preparations were rinsed several times in 0.1 molar cacodylate buffer, postfixed in osmium tetraoxide (2 % O_2O_4 and 0.2 molar cacodylate buffer in ratio 1:1) for two hours, rinsed repeatedly in 0.1 molar cacodylate buffer and dried in an ascending alcohol series (30 - 100 %). For embedding, the preparations were transferred first into the propylene oxide intermedium and after that via a propylene oxide / Araldite mixture in stages of pure Araldite. In the end the embedding and orientation of the preparations in Araldit and the polymerisation at 60 °C in the warming box for three days took place. For a light microscopic selection of relevant structures, first of all, semithin sections of 0.2 µm were cut and stained with toluidine blue. The production of the ultrathin sections was carried out with a diamond knife (Diatome) at the ultramicrotome (Ultracut Leica UCT) at a thickness of 70 nm. The sections were collected from the water surface by vertical passing of the grids (100 mashes) through the water. Biological structures scatter electrons only a little bit since they consist of elements of low atomic number (Nagl, 1981). Therefore the sections were contrasted with heavy metal salts, usually with a uranyl-lead-contrast medium. This contrasting methode with uranyl acetat (Leica Ultrostain 1) and lead citrate (Leica Ultrostain 2) was carried out at a contrasting automat (Leica EM Stain). For examination and documentation the transmission electron microscope LEO 912 Omega (Carl Zeiss NTS GmbH) was used.

2.2.4 Methods: Scanning electron microscopy (SEM)

The scanning electron microscopy enables the three dimensional representation of the surfaces of tissues, cells and parts of isolated cell components. A fine electron beam is distracted so that it scans the preparation. The same scan-generator deflects also the cathode ray in the monitor on which the picture becomes visible (Nagl, 1981). The preparations for the scanning electron microscopy were fixed for two houres with the same glutaraldehyde / paraformaldehyde / cacodylate buffer - mixture as used for transmission electron microscopy. After that the preparations were rinsed repeatedly in 0.1 molar cacodylate buffer and dried subsequently in an ascending alcohol series (30 - 100 %). During

the following "critical-point-drying" (CPDO 30) the critical point is bypassed, that leads to the prevention of contraction and distortion processes while passing a stage boundary (Nagl, 1981). After that the arrangement of the object occurred by means of adhesive film on a carrier plate which was metalised after that with gold-paladium-particles. For the coating of the preparations a sputter coater (Polaron SC 7640) was used. The scanning electron microscopic investigation and documentation occurred with the LEO 1450 VP (Carl Zeiss NT GmbH).

2.3 Indirect Calorimetry

2.3.1 Stages examined

For the investigation of the respiratory abilities of newborn and young *Monodelphis domestica, Macropus eugenii, Mesocricetus auratus, Suncus murinus, Tupaia belangeri* and *Cavia aperea* measurements of the oxygen consumption were carried out. The offspring of *Mesocricetus auratus, Suncus murinus, Tupaia belangeri* and *Cavia aperea* was examined as neonates and at the ages of 4, 7, 11, 14 and 21 days. This experimental outline focuses on the early postnatal developmental stages in which the greatest metabolic changes can be expected, and ends at the age of 21 days where the postnatal development is completed mainly. In addition adult animals were measured for comparison. In *Suncus murinus* in total nine young from three litters and eight adult animals were examined. In *Mesocricetus auratus* at the beginning 12 young, due to infanticide, from the tenth day of life only nine young from two litters and eight adult animals were measured. In *Tupaia belangeri* nine young from four litters were investigated. In *Cavia aperea* initially 11 young, due to the death of one young, from the 11th day of life ten animals from 6 litters were included into the investigation. Measurements of adult *Tupaia belangeri* and *Cavia aperea* could not be carried out with the available system due to their size and were complemented therefore from the literature. Due to the slow postnatal development of the metatherian offspring, the investigation periods in *Monodelphis domestica* and *Macropus eugenii* were extended. In *Monodelphis domestica* the indirect calorimetric measurements were carried out at neonates and young at the age of 4, 7, 11, 14, 21, 28, 42 and 56 days as well as at the adult stage. The young of *Monodelphis domestica* are fixed firmly at the maternal teat for the first 14 to 17 days and a removal from the teat for some time leads to the death of the young. In order to avoid the losses to be expected with single measurements, the young *Monodelphis domestica* were measured together with the mother up to the age of 14 days. Additionally physiological values in the natural environment (body heat and milk supply) can be received with this method. Five litters with sizes of 4, 5, 7, 8 and 11 young were measured. During the investigation period

the number of the measured litters decreased from initially five to three. The reason was infanticide caused by two females. From an age of 21 days 14 young *Monodelphis domestica* were measured separately. In addition 12 adult animals were available for comparative measurements.

In *Macropus eugenii* only single measurements of pouch young of different ages were carried out. The indirect calorimetric measurements took place at the age of 5, 10, 12, 19, 33, 39, 41, 47, 53, 65, 71, 77, 87, 95, 104, 111, 118, 121, 123, 130 and 140 days.

2.3.2 Methods / Techniques

For the measurements to the oxygen consumption of *Monodelphis domestica, Mesocricetus auratus, Suncus murinus, Tupaia belangeri* and *Cavia aperea* cooperation with the research group "Perinatal Adaptation" (Dr. Tzschentke) at the Institute of Biology at the Humboldt University Berlin existed. The metabolic measurements were carried out by means of indirect calorimetry in a half open system (Fig. 4). In this system the animal is trapped in a chamber through which a defined gas volume per unit of time is passing. The oxygen consumption results from the difference of the oxygen content in the inflow and outflow multiplied with the flow rate of the gas flow. The measurements were carried out using an oxygen analyser (Magnos 4; Hartmann & Braun, Frankfurt/Main, Germany) based on the paramagnetic principle. Janke et al. (2002) have already described the experimental methods. Up to three metabolic chambers can be connected with this analyser. Every chamber was supplied with room air by diaphragm pumps. The air flow was controlled and maintained with the aid of

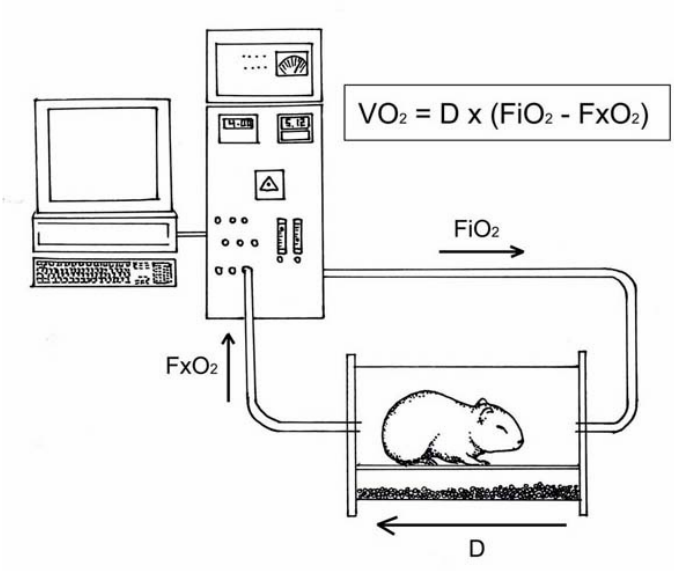

Fig. 4: Principle of indirect calorimetry in an open system. The scheme shows the use of the paragnetic oxygen analyser Magnos 4 (Hartmann & Braun, Germany) and the formula for the calculation of the oxygen consumption (D = flow rate [l/h], FiO_2 = O_2-content in the inhaled gas mixture, FxO_2 = O_2-content in the exhaled gas mixture).

valves. Magnetic valves were used to guarantee the gas flow from the individual chambers to the gas analyser. With a separate pump room air was transfered to the analyser, to calculate the difference between the oxygen concentrations coming from the chamber (after the oxygen consumption of the animal) with that going into the chamber (normal room air). This difference in the oxygen concentration of the in- and outflow was measured in five minutes intervals. Before attaining the analyser the gas flow was dried by passing small silica gel filled tubes. Additionally the gas coming from the chambers was conducted through a membrane filter and a high-sensitive flowmeter. Calibrations were carried out once a month using a gas mixture of a known oxygen concentration.

Waterproof cylindrical metabolic chambers of transparent acryl were used. A smaller chamber with a volume of 290 ml was used for the measurement of neonates and early ontogenetic stages. For the measurement of larger stages and adults a larger chamber with a volume of 1140 ml was used (Fig. 5). Every chamber has two holes in the wall for in- and outflow of the air. The bottom of the chamber was covered with soda lime in order to absorb the carbon dioxide produced during respiration. The oxygen analyser was combined via AD-converter with a personal computer, in order to allow a continuous data recording in five minutes intervals (Software: Labtech Notebook, version 6.3.0; Laboratory Technologies Corporation, Wilmington, USA). The body weight of the animal examined was determined before measuring and fed into the computer, thus the mass specific oxygen consumption was calculated additionally. For the measurements of the oxygen consumption in the tammar wallaby (*Macropus eugenii*) during a research stay in Melbourne, Australia, cooperation with the Department of Physiology at the Melbourne University existed. The measurements were carried out also by means of indirect calorimetry, however, in a closed system. In this case

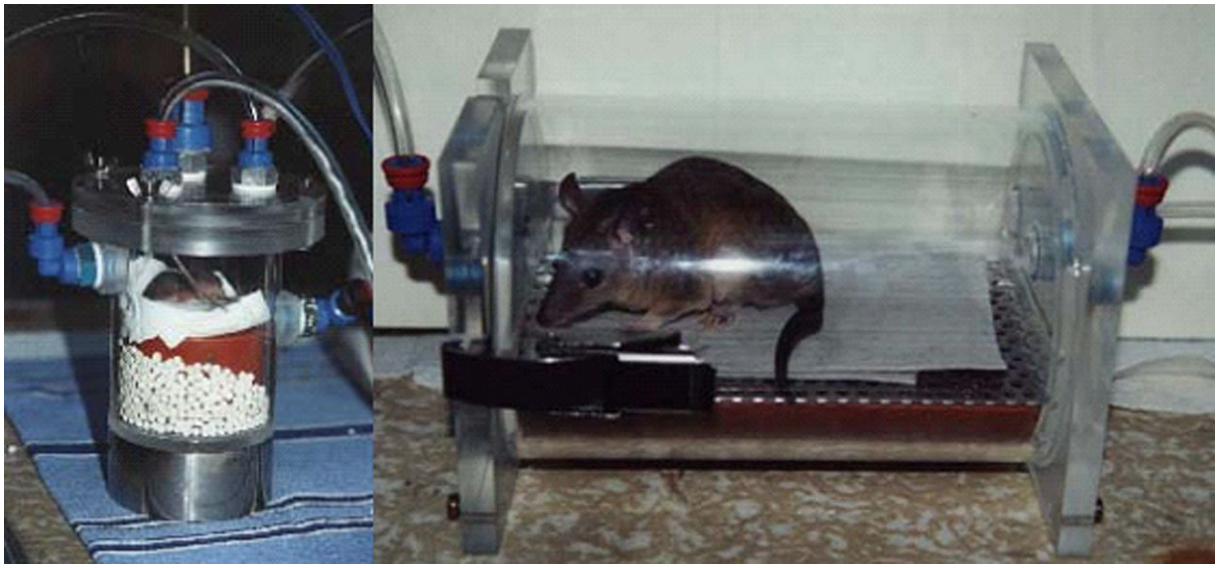

Fig. 5: Applied metabolic chambers. Small chamber has a volume of 290 ml (left). Large chamber has a volume of 1140 ml (right).

the oxygen consumption of an animal is determined from the decrease of the oxygen content in a hermetically sealed metabolic chamber. The principle is based on the comparison of the oxygen concentration in the chamber before the experiment was started and the oxygen concentration after the animal was in the closed chamber for a defined time. This difference of the oxygen content (in %) is related to the chamber volume and the outcome of this is the amount of oxygen consumed by the animal in the chamber (in ml). Related to the body weight and the measured time, the mass specific oxygen consumption (in ml O_2/g/h) is resulting (Fig. 6). The measurement was carried out with a gas analyser (ML 205; AD Instrument's), which was connected with a computer and a corresponding software (Power Lab 4/20). The gas analyser has a sensitivity of 0.1 % and was calibrated with a control gas (constant O_2-content of 16 %) before each experiment. The before described two metabolic chambers from Germany were used. In order to test the system for its reliability and comparability to other systems, 14 days old rats were measured with this system and the results were compared to literature data. The resulting oxygen consumption of 2.67 ml O_2/g/h was reflected very well by the literature values (9 dpn: 2.22; 19 dpn: 2.09 ml O_2/g/h; Sant' Anna and Mortola, 2002) and confirmed the suitability of the system.

2.3.3 Experimental procedure

The respiratory measurements by means of indirect calorimetry (open system) were carried out at a location different from the animal facility. The young had to be transfered to the experimental location. To avoid this stress of transportation, in the smaller species the

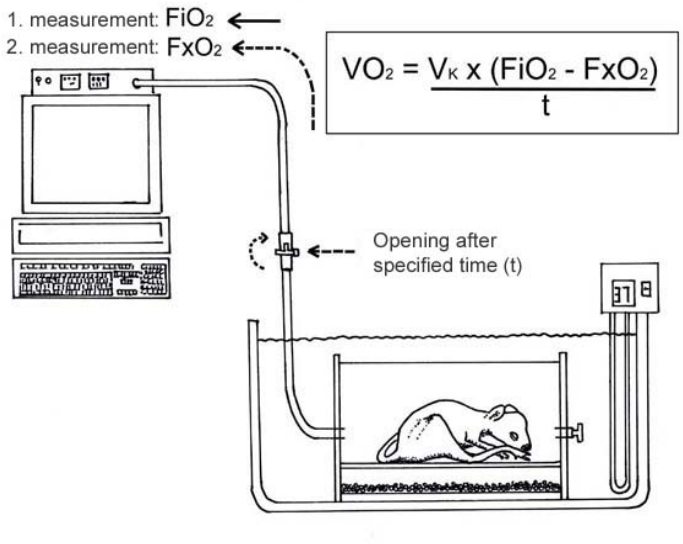

Fig. 6: Principle of indirect calorimetry in a closed system. The scheme shows the use of the gas analyser ML 205 (AD instrument's) and the formula for the calculation of the oxygen consumption (V_K = volume of the chamber, FiO_2 = O_2-content in the chamber before the measurement, FxO_2 = O_2-content in the chamber after the measurement, t = time of measurement).

pregnant females of *Monodelphis domestica, Mesocricetus auratus* and *Suncus murinus* were transported to the experimental workstation before parturition and kept there for the duration of the series of experiments. Young of *Tupaia belangeri* and *Cavia aperea* were transferred from the animal facility to the experimental location in an isolated container to reduce thermal stress. During the measurements ambient temperatures of 27-28 °C were provided, according to the thermoneutral zone of the species examined. Immediately before the measurement the body weights of the animals were determined and fed into the computer for the calculation of the mass specific oxygen consumption. For the measurement of the neonates and early ontogenetic stages one to three young of a litter were taken from the nest and placed separately into one of the three small metabolic chambers (290 ml). For the measurement of older stages, adults and larger species, such as *Cavia aperea*, the large metabolic chamber (1140 ml) was used. The chambers were hermetically sealed before the measurement started. The gas flow rates for the chambers were regulated to a constant value. The chosen flow rate depended on size and age of the stages examined. For *Mesocricetus auratus, Suncus murinus* as well as for the single measurements of *Monodelphis domestica* (from 21 dpn) at the beginning of the series of experiments flow rates of 4 l/h were set in order to achieve a higher sensitivity in these small stages. During the postnatal development and in adults the flow rate was increased to 9 l/h (peak value of the system). The total time of measurement was 1.5 hours. After an adaptation phase of 15 to 30 minutes the data were recorded during a one-hour measuring period. During the measurement the current oxygen consumption could be observed by the volume percent displayed on the monitor. Additionally the activity of the measured animal was observed. To guarantee the comparability of the data standard metabolic rates (SMR) were measured. The conditions for the SMR included that the organism was rested (or as near to rested as is possible) and in a thermoneutral environment. In general the animals investigated calmed down quickly and in particular young often fell asleep. For the documentation the data were printed as well as transmitted to a computer and processed using the software Microsoft Excel. From the measured data of animals of the same age class the mean value and standard deviation were computed. As already mentioned in 2.3.1, the young *Monodelphis domestica* were measured together with the mother up to the age of 14 days. In female *Monodelphis domestica* the standard metabolic rate is nearly the same during pregnancy and early lactation (Harder et al., 1996; Hsu et al., 1999). For that reason, first of all, the oxygen consumption of the chosen female was measured during the late pregnancy and the result was taken as a comparative value for the postpartal measurements. This value was diverted from the common measurement of the female with their young and the oxygen consumption of the young was determined. Considering the respective litter size the oxygen consumption per young was computed. As control, that in fact the oxygen consumption of the

young was measured, after the common mother-young measurement a control measurement with detached, separated young was carried out at the age of 21 days. The results of both methodes show a concordance of the oxygen consumption of the young (common: 0.433; separately: 0.608 ml O_2/g/h). Equal values from single measurements of *Monodelphis domestica* at the ages of zero to nine days give an additional confirmation of the applicability of the common mother-young measurements (Singer, 2001). However, for this method a high standardised test procedure is necessary. Due to the low body weight of the young, some factors can strongly influence the result, for example, a weight increase of the female after feeding. The respiratory measurements of pouch young of the tammar wallaby (*Macropus eugenii*) in Australia depended on the available ontogenetic stages during the research stay of seven weeks. On the day of investigation the respective females with the selected pouch young were caught from the yards of the research colony and transported to the university. Before starting the experiment a calibration of the gas analyser, the warming of the water bath to 37 °C and a control measurement of the empty system for 15 or 30 minutes were performed. After completion of the experimental preparations the female was fixed, the pouch young was detached from the teat and taken from the pouch. The age was determined by head length measurements. The data of the head length were compared to the growth curves of Poole et al. (1991) to estimate the age. After that the pouch young was kept moist and warm and was transfered to the close experimental location. The measurements of the pouch young up to the age of 65 days were carried out in the small metabolic chamber (290 ml). For the older developmental stages the large metabolic chamber (1140 ml) was used. The pouch young was placed into the metabolic chamber on cellulose, the lid was closed airtight and the chamber set into the 37 °C warm water bath. Through this ambient temperature the heat in the pouch of the female was simulated. By means of a three-way-valve the connection of the chamber to the gas analyser was interrupted. Pouch young up to the age of 19 days were measured for 30 minutes (for quantifiable oxygen consumption); older stages remained for 15 minutes in the closed system. After that the three-way-valve was opened and the current oxygen concentration in the chamber was determined by the gas analyser. Through the comparison of the first measured initial oxygen concentration (comparative value) with the final oxygen concentration (after 15 or 30 minutes) in the chamber, the oxygen consumption of the pouch young was calculated. After finalisation of the measurement the pouch young was taken from the metabolic chamber. It was returned immediately to the female and was reattached to the teat (see Renfree and Tyndale-Biscoe, 1978). All animal experiments were approved by the Animal Experimentation Ethics Committees (registration number G 0249/02).

2.4 Phylogenetic reconstruction

To understand evolution, it is necessary to know not only the character states of living organisms, but also of their ancestors. An increasingly popular method is to infer ancestral character states by mapping the character states of living organisms onto phylogenies using the method of maximum parsimony (Cunningham, 1998). Reconstruction of morphotypes follows the algorithm for reconstructing ancestral states using parsimony (Zeller and Freyer, 2001). As pointed out by Cunningham et al. (1998), the algorithm uses a “downpass” and “uppass” traversal to optimise ancestral states using two rules: Rule 1: if descendant nodes share any states in common, assign the set of shared states to the ancestor; Rule 2: if no states are shared in descendant nodes, assign the union of descendant’s states to ancestor. Whereas the downpass optimisation proceeds “down” the tree towards the root, the uppass optimisation proceeds “up” the tree away from the root both optimising each ancestral node. In a final optimisation, the sets of downpass and uppass reconstruction are optimised to the state that has the greatest number in both reconstructions (Zeller and Freyer, 2001). The outgroup changes as does the respective ingroup during the process of optimising. This procedure is conducted using the cladograms by Luckett (1994) for Marsupialia and by Springer et al. (2003) for Placentalia.

3 Results

3.1 General postnatal development

For a holistic view of the different reproductive strategies of marsupials and placentals a comparison of the general degree of development of their offspring is essential. For this reason a description of the external characteristics of the neonates and the postnatal development of the species examined are presented in this chapter. The crown-rump-lengths (CRL) of the animals, mentioned in the text, are single measurements. The presented body weights are mean values of several animals (see table II of the appendix). Growth rates are presented with mean values and standard deviation.

3.1.1 Characteristics of neonates

A first look at the neonates of Monodelphis domestica, Macropus eugenii, Tupaia belangeri, Suncus murinus, Mesocricetus auratus, Cavia aperea and Macroscelides proboscideus reveals strong differences concerning the external appearance (Fig. 7). An additional view at histological sections through the anterior abdominal region shows also remarkable differences concerning the internal anatomy between marsupial and placental neonates (Fig. 8).

The marsupial neonates are born after a relatively short gestation period, 14 days in Monodelphis domestica and 29 days in Macropus eugenii. The appearance of the newborn gray short-tailed opossum (Fig. 7a) and the newborn tammar wallaby (Fig. 7b) is embryonic. The posterior part of the body is curled to the ventral side. The newborn Monodelphis domestica has a CRL of 10 mm and a weight of 100 mg. The neonatal Macropus eugenii has a CRL of 13 mm and a weight of 370 mg. Both neonates appear to be extremely altricial, the naked skin is very thin and transparent. Blood vessels and internal organs are visible under the skin. The newborn marsupials have an unspecialised peri-oral region with relatively small nasal swellings surrounding the prominent nostrils. In the newborn Monodelphis domestica, the oral opening and the nose form together the oral shield. The oral shield is closely apposed to the skin around the base of each teat, ensuring a firm attachement to the teat, an important necessity in this pouchless species. The eyelids of both newborn marsupials are slightly visible but closed. The eye primordia bulge from the head and contain a prominent ring of retinal pigmentation. The ear primordia are visible, but the later ears are only recognisable as small bright fields on both sides of the head. Immediately after birth the marsupial neonates climb actively from the urogenital opening to the teats. In this connection

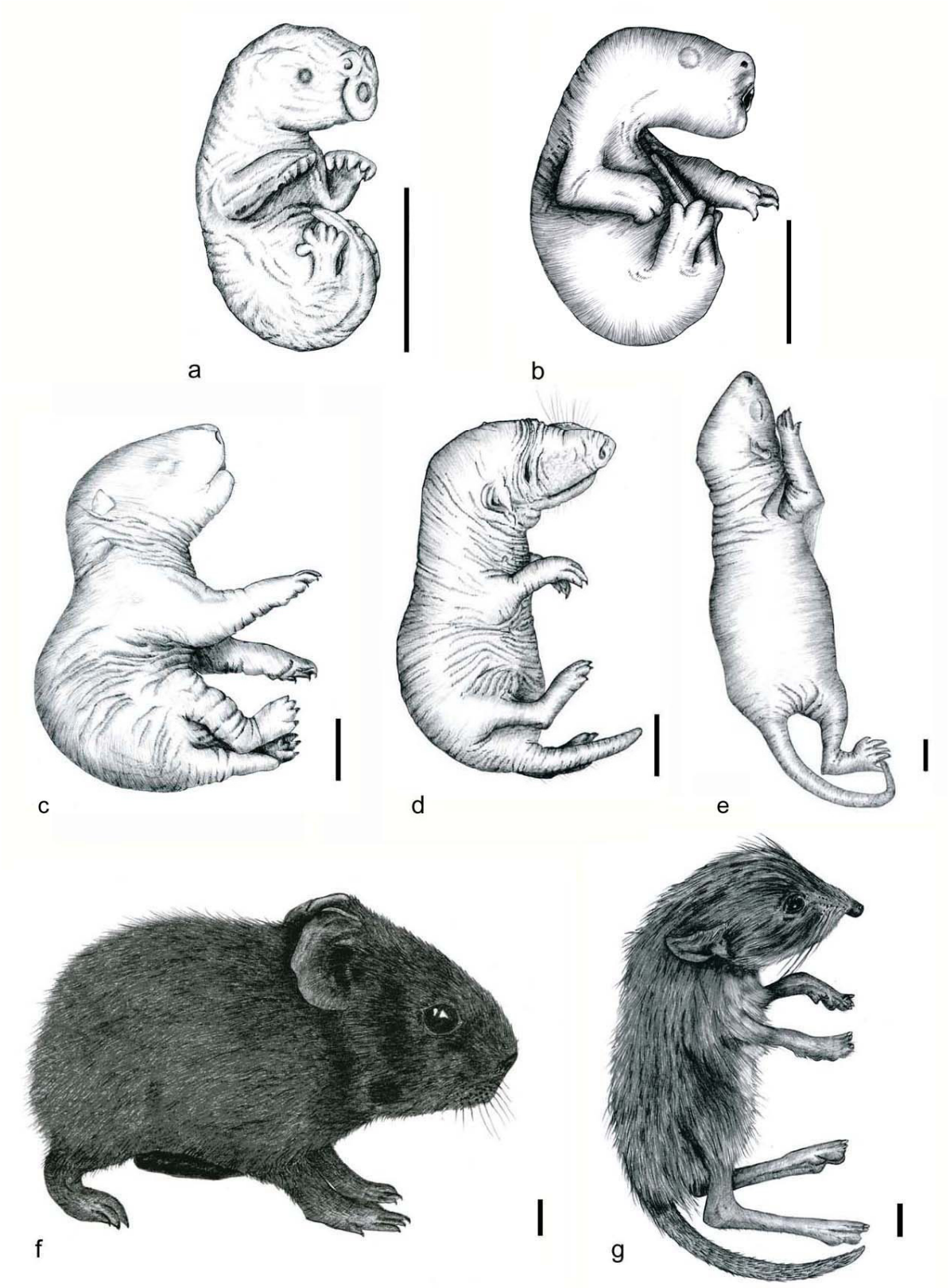

Fig. 7: Original drawings of neonatal *Monodelphis domestica* (a), *Macropus eugenii* (b; redrawn from Hughes & Hall, 1988), *Mesocricetus auratus* (c), *Suncus murinus* (d), *Tupaia belangeri* (e), *Cavia aperea* (f) and *Macroscelides proboscideus* (g). The appearence of the two newborn marsupials (a, b) is embryonic with a not well differentiated oro-nasal region; note also the more advanced forearms and the undifferentiated hindlimb paddles. The appearence of the altricially born eutherians (c, d, e) is significantly more advanced with well developed extremities including claws, but they are naked and eyes and ears are closed. The appearence of the precocially born eutherians (f, g) is most advanced with open eyes and ears and well developed fur. Scale bar = 0.5 cm.

the differences in the external morphology of the forelimbs and the hindlimbs can be explained. In the newborns of *Monodelphis domestica* and *Macropus eugenii* well developed and precociously pronated forelimbs are present. These advanced forelimbs perform sinous contractions with which the neonate climbs actively through the fur of the female to the teat. They must have the capacity for digito-palmar prehension in order to cling in the fur. The terminal ends of the forelimb digits appear “blade-like” and are finely pointed with recurved deciduous claws. The hindlimbs by contrast are unrotated paddles without claws. They project at right-angles to the main axis of the body and are retarded in their development when compared to the forelimbs. A histological section of the newborn *Monodelphis domestica* (Fig. 8a) gives a first look on the internal structure. Internally the skeleton of *Monodelphis domestica* is provided with a complete complement of cartilaginous elements. The already decribed movement of the advanced forelimbs requires a well developed musculature. In fact, the brachial plexus is prominent in the marsupial newborn. The sinous locomotory movements of the torso are based on muscle fibres that arise from the cervical to the lumbar region.

The newborns of Mesocricetus auratus, Suncus murinus and Tupaia belangeri represent the altricial placental type (Fig. 7 c, d, e). All three neonates are more advanced than the marsupial neonates, even the newborn Mesocricetus auratus, although the gestation period of 16 days is nearly the same as in Monodelphis domestica. The gestation periods of Tupaia belangeri (45 days) and Suncus murinus (31 days) are significantly longer. The newborns of Mesocricetus auratus and Suncus murinus have similar birth weights and body lengths. The golden hamster has a CRL of 30 mm and a weight of 2.1 g and the musk shrew has a CRL of 35 mm and a weight of 2.8 g. The newborn Tupaia belangeri is larger and heavier; it has a CRL of 60 mm with an additional tail length of 43 mm and has a weight of 18.3 g after first weaning. The bodies of all three altricial placental neonates are hairless but definitely more shaped than the bodies of the neonatal marsupials. The skin appears pink and transparent and the internal organs are shining through the thin skin. The oro-nasal region is well differentiated. In Mesocricetus auratus incisors are already present at birth, in Suncus murinus and Tupaia belangeri the teeth are short before eruption. The eyelids of all three neonates are closed, but the eyes seem to be well developed. In newborns of Tupaia belangeri the blinking reflex is present at birth. At the time of birth the auditory canal is closed but the pinnae are not adnated with the head skin and clearly visible. The rhinarium is surrounded by small vibrissae. In Tupaia belangeri vibrissae are recognisable also at the ventral and dorsal body surface. A special feature in Tupaia belangeri is a patagium that extends from the thighs to the upper arms and from there to the neck. It becomes visible at spreading out the extremities. Although the altricial newborns are unable to move coordinately and are nidicolous, fore and hind limbs are at the same advanced stage of

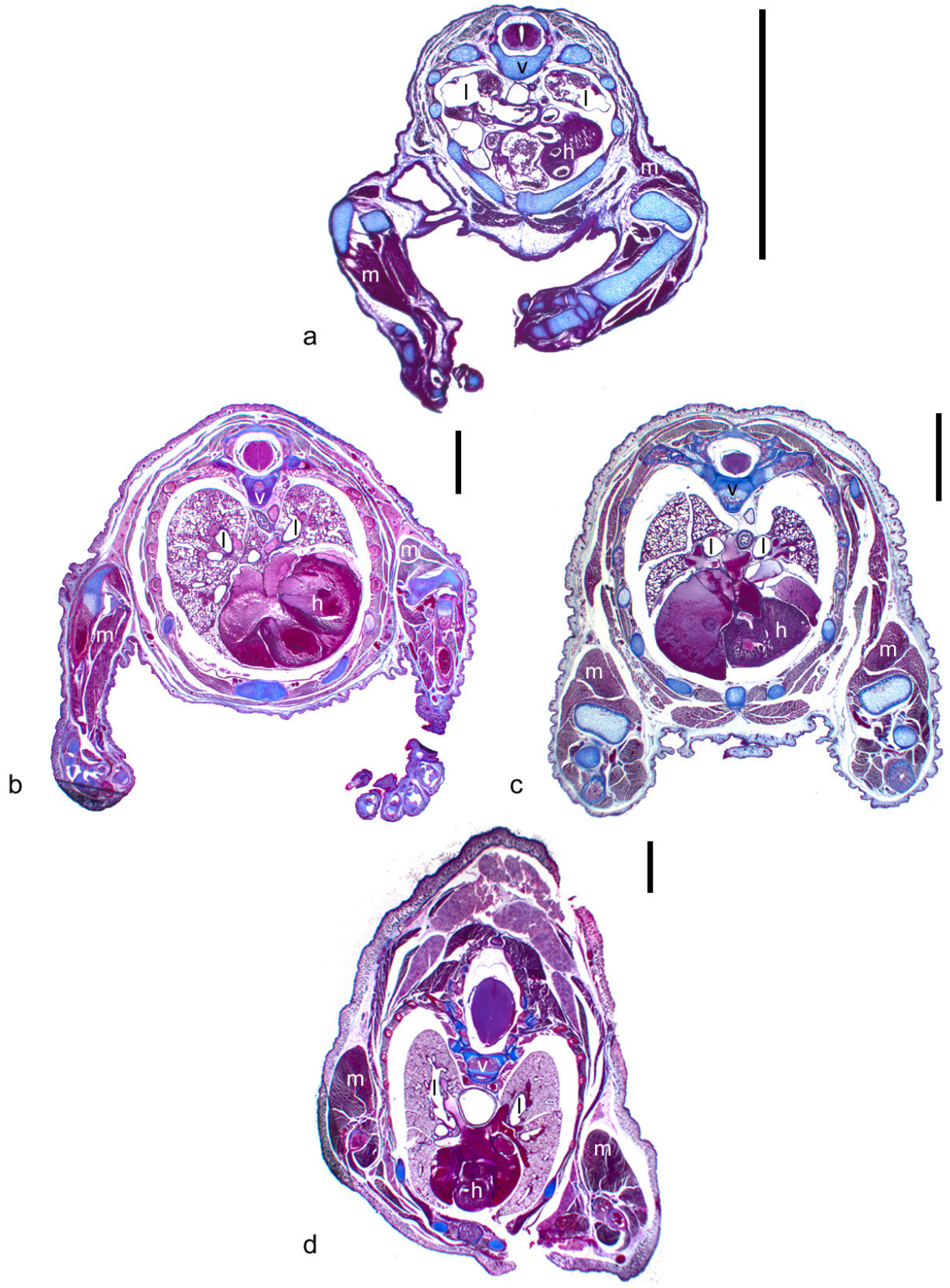

Fig. 8: Light micrographs of histological sections through the anterior abdominal region of the neonatal *Monodelphis domestica* (a), *Suncus murinus* (b), *Mesocricetus auratus* (c) and *Macroscelides proboscideus* (d). The internal structure of *Monodelphis domestica* shows a small undifferentiated heart; the whole skeleton is of cartilage and only a few muscles are present at birth. The newborn *Suncus murinus* and *Mesocricetus auratus* feature a differentiated heart, vertebrae start to ossify and muscles are distinctive. The newborn *Macroscelides proboscideus* is most advanced, with a well differentiated heart. The skeleton is completely ossified and extremities and dorsum are extremely muscular. h, heart; l, lung; v, vertebra; m, muscles. Azan staining. Scale bar = 0.5 cm.

development and the digits already show claws. At birth *Tupaia belangeri* shows an orientation movement, a rotation with the whole body that arise from crawling movements of the front legs. The hind legs are hardly involved, head and belly remain on the ground. The histological sections of the newborn *Suncus murinus* (Fig. 8 b) and *Mesocricetus auratus* (Fig. 8 c) allow an insight into the internal anatomy of altricially born placentals. The skeleton of the limbs and digits consists of cartilage, but histological sections through the ribs and the vertebrae show first ossifications. Musculature is present but due to the immobile behavior in the nest it is less developed.

The newborns of *Cavia aperea* and *Macroscelides proboscideus* represent the precocial placental type (Fig. 7 f, g). In comparison with the previously described neonates these two precocially born placentals are most advanced in their development. The gestation period in *Cavia aperea* and *Macroscelides proboscideus* is 61 days. The newborns are relatively larger and heavier than those of marsupials and altricial placentals. *Cavia aperea* has a CRL of 80 mm and a weight of 60.5 g at birth. The newborn *Macroscelides proboscideus* has a CRL of 60 mm and a weight of 6-8 g. The external appearance is similar to the adults; however, they appear more compact. They are born already covered with fur, vibrissae are present. Eyes and ears are open at the time of birth and all sense organs are fully developed. All four extremities are advanced and enable coordinate locomotion shortly after birth. The digits of the fore- and hindlimbs are provided with claws. Teeths are present at birth and in particular *Cavia aperea* is feeding solid food from the first day of life. A histological section through the anterior abdominal region of *Macroscelides proboscideus* shows complete ossified skeletal elements (Fig. 8 d). Although the newborn short-eared elephant shrew is philopatric and is visited regular by the female, the young is able to move fast. For this advanced locomotory behavior a well developed musculature is necessary. In fact extremities and dorsum are relatively muscular.

The postnatal development of the six species examined is described in the next chapter.

3.1.2 Postnatal development

3.1.2.1 Monodelphis domestica

The postnatal development of *Monodelphis domestica* proceeds slowly. A look at the growth curve of *Monodelphis domestica* shows a slow weight increase during the first five weeks of life, but from this time the increase in weight is more rapidly (Fig. 9). Corresponding to the initially low growth rate a slow morphological development takes place (Fig. 10). At the age of eight days the young *Monodelphis domestica* has a CRL of 13 mm and a weight of around 440 mg (Fig. 10 b). The posterior part of the body is still rolled up to the ventral side and the

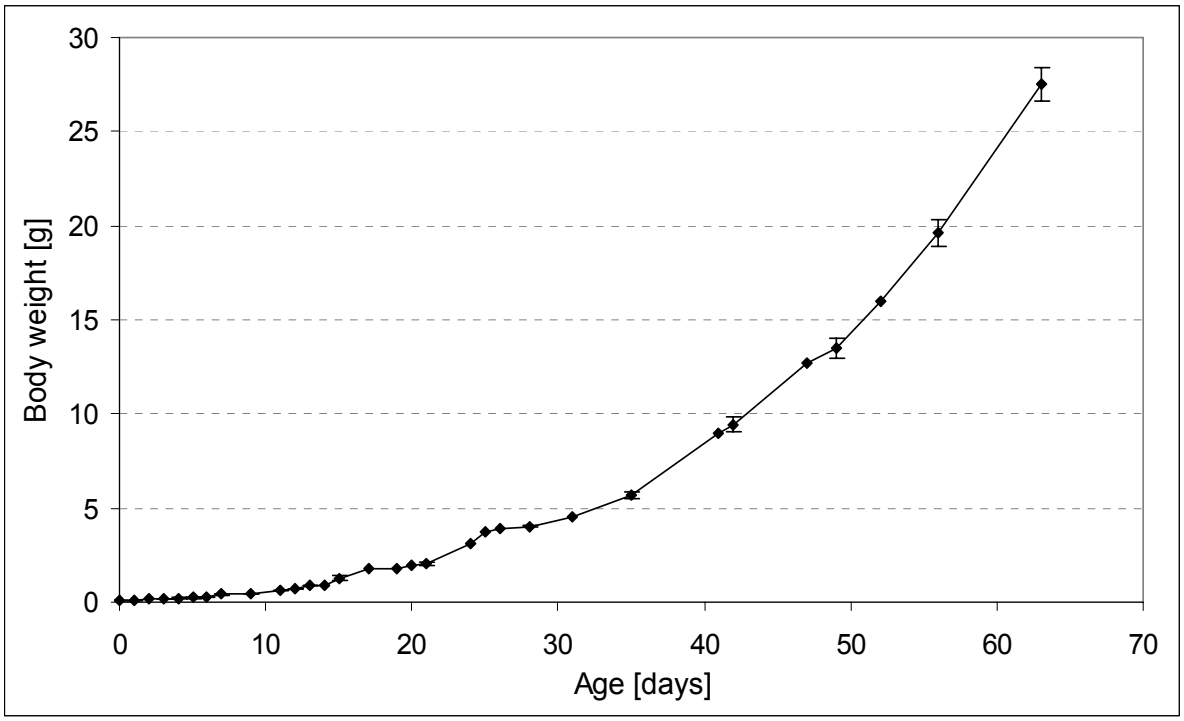

Fig. 9: Growth curve of *Monodelphis domestica* during the first nine weeks of life

eyes and ears are closed. The pinnae are not differentiated, but already recognisable by a fold. The oro-nasal region starts to differentiate and the oral fissure is visible by a fold. In the hindlimbs no claws exist. At the age of 14 days *Monodelphis domestica* has a weight of 900 mg and a CRL of 20 mm (Fig. 10 c). The posterior part of the body stretches to the dorsal side, thus the back is straightened. The iris is clearly visible under the skin; however, the eyes are still closed. The palpebral fissures of the eyelids are visible. Now the pinnae are fully developed and the ears are open. The oro-nasal region is in progress, the oral fissure is completely formed and open. The period of permanent attachment at the female's teat is finished at this time and the young may be left alone in the nest. However, young are frequently present on the teats during the third week of life. The first fine hairs and vibrissae appear at the head. Now claws are present at the hind limbs. The fore and hind limbs are nearly at the same developmental stage. At the age of 21 days *Monodelphis domestica* has a weight of 2.1 g and a CRL of 30 mm (Fig. 10 d). Eyes are still closed. A darker pigmentation becomes visible at the back. Now the young are often lying in a nest and are left by the female for a short time. At the age of 28 days the young have a CRL of 35 mm and a weight of 4.0 g (Fig. 10 e). The eyes are open and the fur is growing. Between 28 and 35 days the young start to feed solid food. With 56 days *Monodelphis domestica* has a weight of 19.6 g and a CRL of 69 mm (Fig. 10 f). Compared to the earlier stages the appearence is more like an adult, the head is elongated and the body is completely fured. At the age of eight weeks *Monodelphis domestica* is weaned. The sexual maturity of both sexes is reached between 120 and 140 days of life.

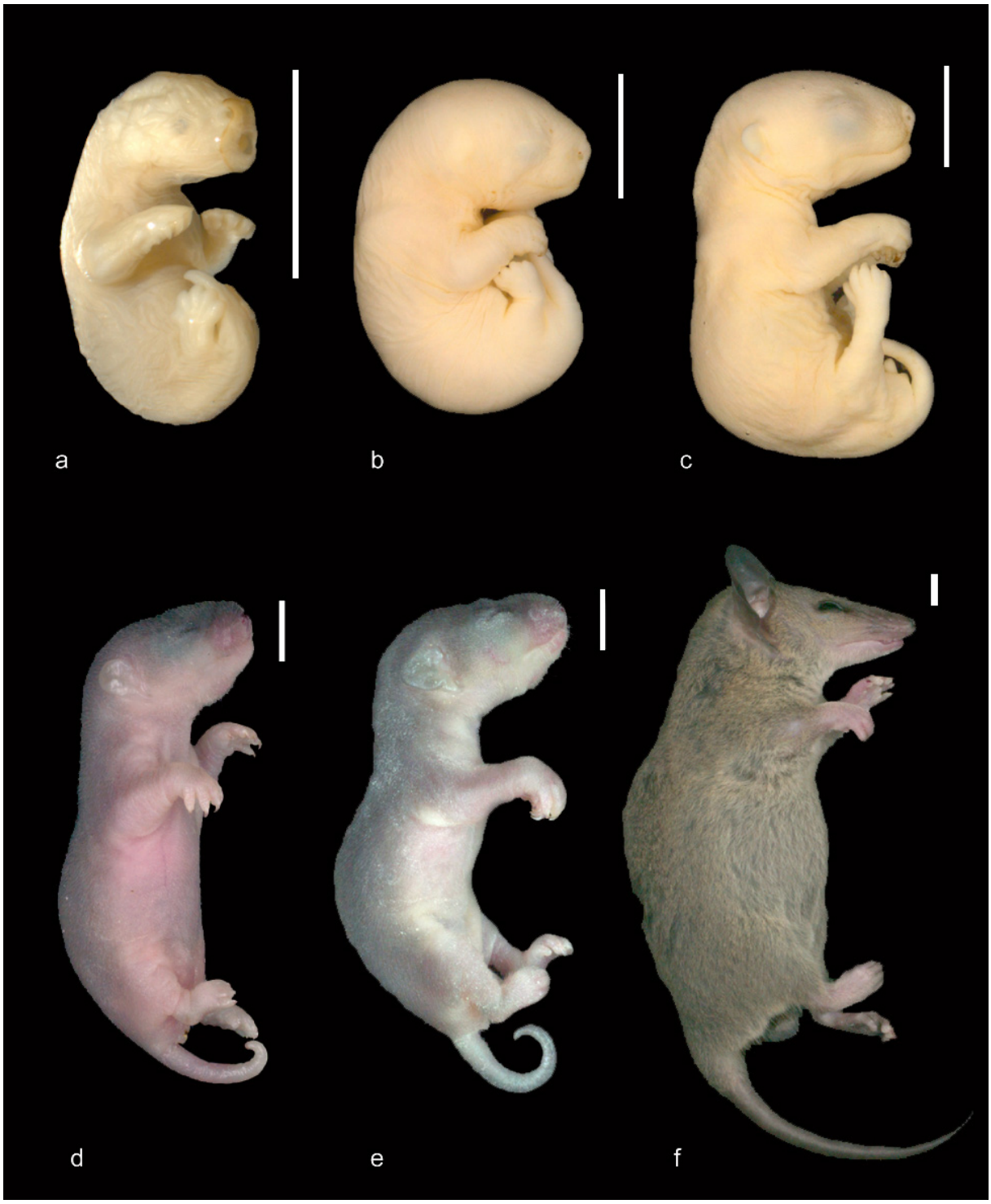

Fig. 10: Postnatal development of *Monodelphis domestica*. Lateral view of young at day 0 (a), 8 (b), 14 (c), 21 (d), 28 (e) and 56 (f). Scale bar = 0.5 cm.

3.1.2.2 Macropus eugenii

The tammar wallaby *Macropus eugenii* shows a slow postnatal growth rate during early pouch life (Fig. 11). The growth curve resembles that of *Monodelphis domestica*. Up to 47 days of life the weight increase is slowly, from that time the growth rate rises. Also the

morphological development proceeds very slowly in *Macropus eugenii* (Fig. 12). At the age of five days the pouch young has a CRL of 21 mm and a weight of around 870 mg (Fig. 12 a). The posterior part of the body is still rolled up ventrally and the eyes and ears are closed. Eyelids are still absent, but the iris is visible under the thin skin. The ears are still closed and pinnae are not developed yet. An oral shield is present and the oro-nasal region is undifferentiated. At this time, the extremities show a strong difference in the degree of development. The forelims are advanced, but the hindlimbs are retarded and claws are missing. With 19 days the pouch young *Macropus eugenii* has a CRL of 27 mm and a weight of 3.1 g (Fig. 12 b). The posterior part of the body stretches to the dorsal side. Eyes and ears are still closed, but the palpebral fissures of the eyelids are visible and pinnae start to differentiate. The oro-nasal region is elongated and reveals already the final form. The oral fissure is not present yet, but visible by a fold. At the age of 41 days *Macropus eugenii* has a CRL of 49 mm and a weight of 10.6 g (Fig. 12 c). The back line is further straightened. The hindlimbs are elongated and the digits have claws. They have reached the same developmental stage as the forelimbs. Eyes and ears are still closed. Pinnae are clearly visible. The oral fissure is completely developed and open. At the age of 65 days the pouch young has a weight of 27.6 g and a CRL of 75 mm (Fig. 12 d). The whole body, but especially the hindlimbs, increase in length. With 87 days the pouch young has a weight of 45.4 g and a CRL of 82 mm (Fig. 12 e). The nasal region gets darker in colour and vibrissae

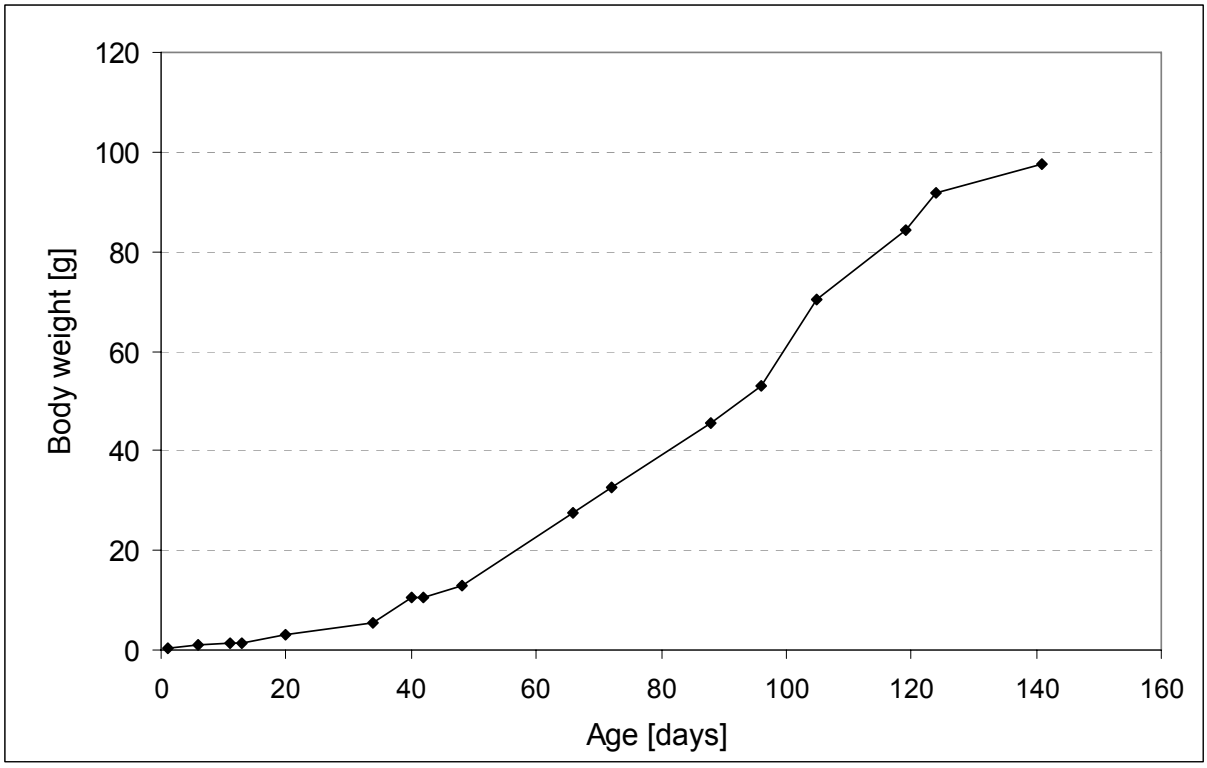

Fig. 11: Growth curve of *Macropus eugenii* during the first 20 weeks of life

Fig. 12: Postnatal development of *Macropus eugenii*. Lateral view of young at day 5 (a), 19 (b), 41 (c), 65 (d), 87 (e), 104 (f), 123 (g) and 140 (h). Scale bar = 1 cm.

around the nose are distinctive. At the age of 104 days *Macropus eugenii* has a weight of 70.3 g and a CRL of 95 mm (Fig. 12 f). The ears are pigmented and start to unfold, they

seem to be open. Now the permanent adhesion at the teat is finished and the pouch young comes off for the first time. With 123 days the pouch young tammar wallaby has a weight of 91.9 g and a CRL of 106 mm (Fig. 12 g). The snout is coloured dark. At the age of 140 days *Macropus eugenii* has a CRL of 121 mm and a weight of 97.7 g (Fig. 12 h). With 140 days the eyes open. The further development of *Macropus eugenii* is described by Tyndale-Biscoe and Janssens (1988) and Janssens et al. (1997). By day 160 the young is able to stand unaided and the pelage is thickening. Setchell (1974) states that with 180 days thyroid functions appear to be fully developed and the pouch young is homeothermic. Although the young puts its head out of the pouch and nibbles gras at about 180 days, it does not make its first excursion from the pouch until about day 190. The young starts feeding from this time, but it returns to the female to suck for several more weeks. Herbage progressively forms a greater portion of the diet. The young *Macropus eugenii* leaves the pouch permanently at about 250 days and ceases to suck around 270 days. The onset of sexual maturity is in females at the age of 8 months and in males with 24 months (Tyndale-Biscoe and Renfree, 1987).

3.1.2.3 Mesocricetus auratus

The golden hamster *Mesocricetus auratus* is an altricially born Placentalia and represents the large mammalian order Rodentia. The postnatal growth curve *of Mesocricetus auratus* shows a sigmoid course of the curve (Fig.13). The growth rate can be subdivided into four stages. During the first eight days the daily weight increase averages 0.7 g. This seems to be a comparatively slow growth rate. In the following period between 9 and 14 days the mean weight gain is increased to 1.4 g per day. The highest daily weight increase is reached between 14 and 28 days and averages 3.1 g. Afterwards the weight increase is decelerated to 0.9 g per day. The morphological development proceeds rapidly (Fig. 14).

At the age of four days *Mesocricetus auratus* has a weight of 5.0 g and a CRL of 40 mm (Fig. 14 b). The eyes are still closed, but the eyelids are clearly visible. The ears begin to open with four days. For the first two to four days the pinnae are in hinged position. With four days the dorsal pinnae margins are free from the head skin. The skin at the back and the head is dark pigmented. The ventral parts of the body, the belly, the muzzle and the extremities are noticeable less pigmented. At day 4 *Mesocricetus auratus* is fine haired and at the flanks two bright spots are visible. These spots indicate the later position of the lateral glands, which are present from day 25. At this age the locomotion is performed by crawling movements and is still uncoordinated. With seven days the young has a weight of 7.1 g and a CRL of 45 mm (Fig. 14 c). The eyelids are dark pigmented and the palpebral fissure is deep imprinted, but the eyes are still closed. The process of furing proceeds. The colouration of skin and fur

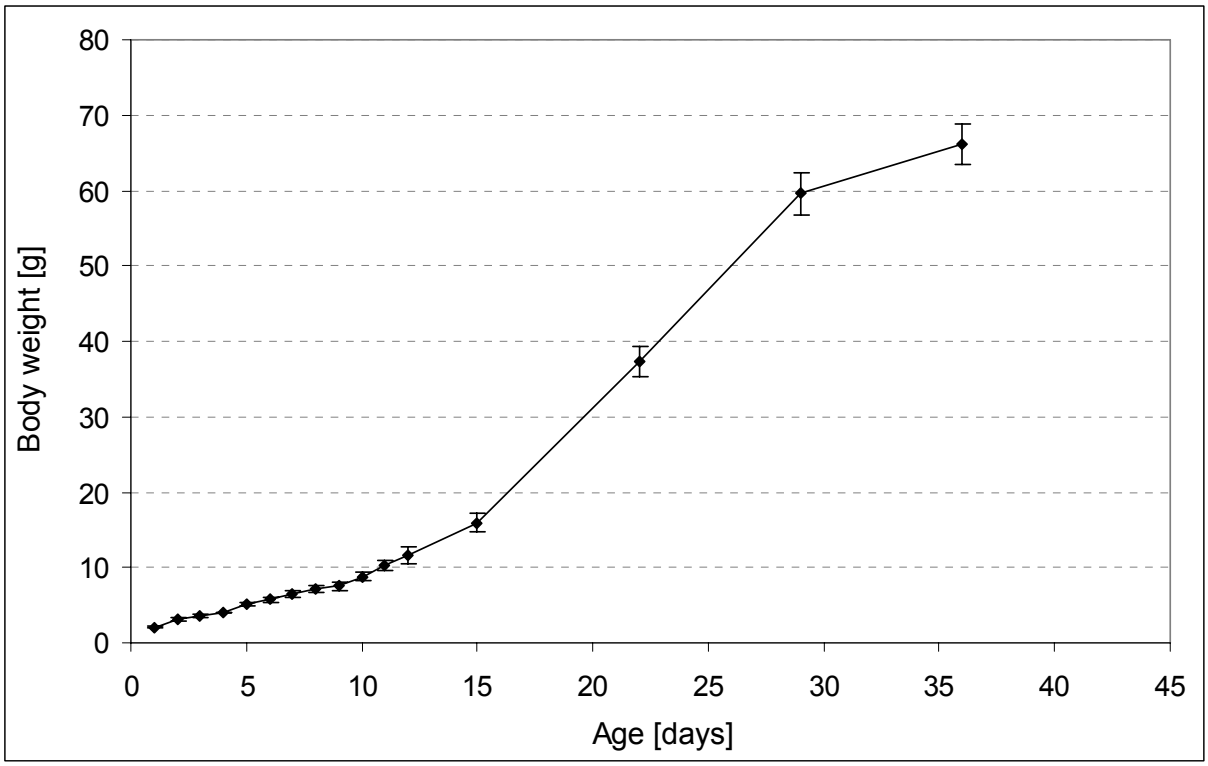

Fig. 13: Growth curve of *Mesocricetus auratus* during the first five weeks of life

remains constant, dark at the dorsal and bright at the ventral parts of the body. Additionally a coloured onset appears around eyes and muzzle. The facial vibrissae are clearly visible but still colourless. At the age of 11 days *Mesocricetus auratus* has a weight of 11.6 g and a CRL of 58 mm (Fig. 14 d). Now the skin pigmentation decreases in colour intensity constantly and the coloured hair, especially in head, neck, shoulders and thighs increases. With 11 days the whole back fur is coloured orange and appears longitudinal striped with some black guard hair. On the ventral body side a collar is developed. The incisors are already present at birth, but there is a diastem of 1 mm between the upper incisors. This tooth gap is closed now, so that both incisors stand close to each other. Besides, the first molar is breaking through. From this time the young starts to feed solid food. The young is now able to coordinate locomotion. At the age of 14 days the young has a weight of 15.9 g and a CRL of 61 mm (Fig. 14 e). The eyes start to open between 11 and 13 days and are already open at the age of 14 days. The fur is still shorter than that of adults but also dense and wooly. The young becomes more active now and examines the surrounding of the nest. With 21 days *Mesocricetus auratus* has a weight of 37.2 g and a CRL of 81 mm (Fig. 14 f). The fur increases in length and is similar to that of adults in colour and structure. In addition to the first molar also the second and the third molar are present now. This time is associated with the beginning of independence and weaning of the young. The initiation of sexual maturity is with four to five weeks in both sexes (Puschmann, 2004).

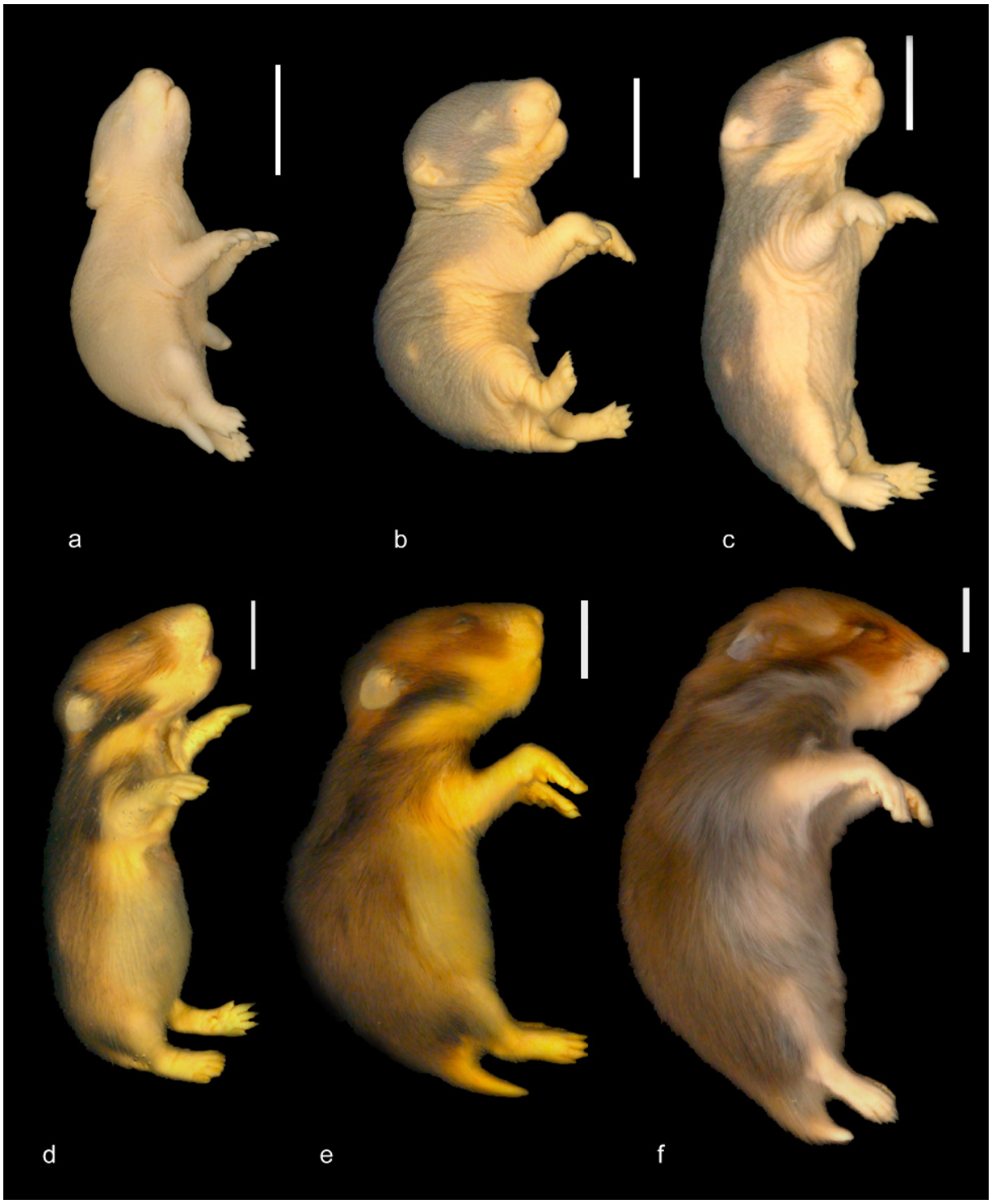

Fig. 14: Postnatal development of *Mesocricetus auratus*. Lateral view of young at day 0 (a), 4 (b), 7 (c), 11 (d), 14 (e) and 21 (f). Scale bar = 1 cm.

3.1.2.4 Suncus murinus

The second altricially born Placentalia, included in this study, is the musk shrew *Suncus murinus.* The postnatal growth curve shows a rapid gain in weight (Fig. 15). During the first 28 days a linear increase of the body weight can be observed. The daily weight gain is in average 1.6 g. After that time the adult weight is reached and the young are full-grown.

According to the rapid growth rate also the morphological development proceeds rapidly (Fig. 16). At the age of four days *Suncus murinus* has a weight of 7.8 g and a CRL of 53 mm (Fig. 16 b). Eyes and ears are still closed. The dorsal skin at back and head assume a grey cast but devoid of underfur. The belly is still pink. Lower limbs, the middorsal muzzle, and the tail are darker in colour than the other body parts. The facial vibrissae and body guard hairs are clearly visible but still colourless. The lateral gland sites become visible and appear as hairless oval spots. The locomotion is still uncoordinated and consists of crawling movements. The muscular coordination and mobility is steadily improved during the first days of life. With seven days *Suncus murinus* has a weight of 13.2 g and a CRL of 62 mm (Fig. 16 c). The eyes are still closed. The pinnae are clearly more differentiated and the dorsal pinnae margins are free from the head skin. At this age stage the auditory canal appears to be open. With seven days, soft, grey to dark underfur is present. The skin of the muzzle, tail and lower limbs is dark coloured, the dorsum appears medium to dark grey, but the belly is noticeably less pigmented. Numerous colourless vibrissae are present at the head, in particular around the nose. At the age of 11 days the young has a weight of 20.8 g and a CRL of 75 mm (Fig. 16 d). The underfur increases in length and becomes velvety. Nearly all the vibrissae are bicoloured with dark bases and light grey or colourless tips. The eyes start to open with eight to nine days and are fully open at day 11. Between 8 and 10 days after birth the eruption of

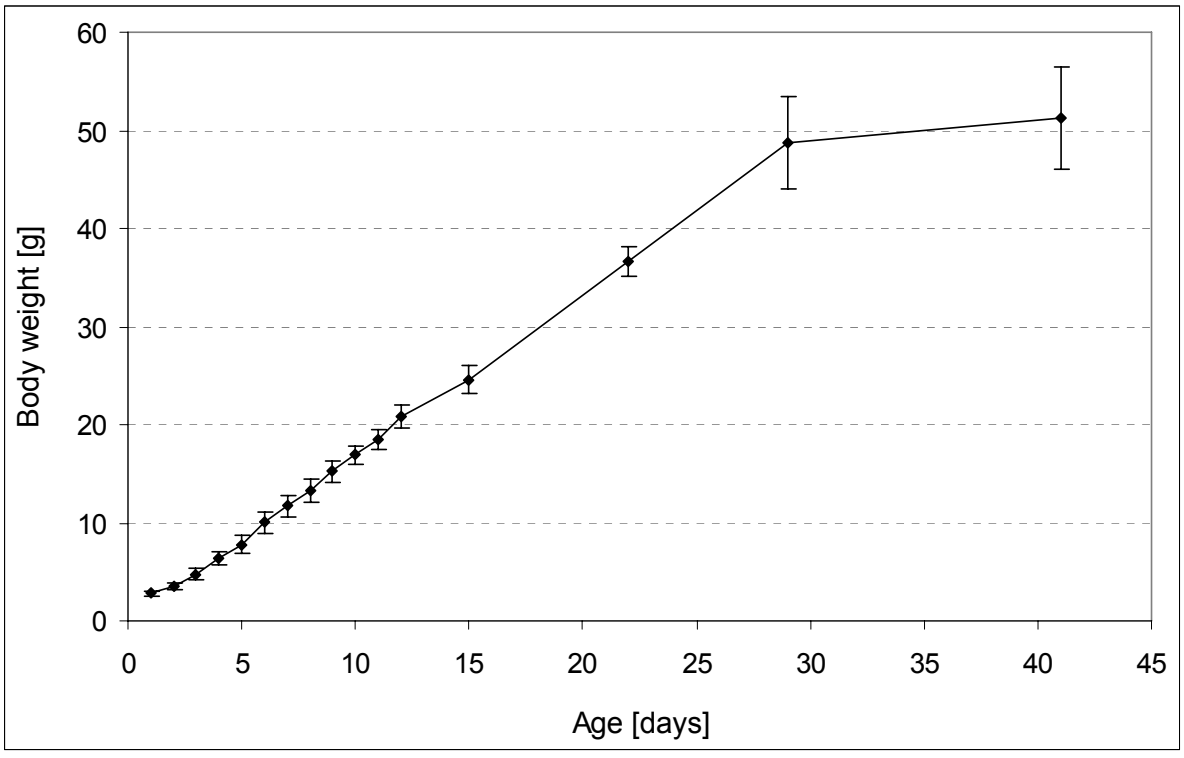

Fig. 15: Growth curve of *Suncus murinus* during the first six weeks of life

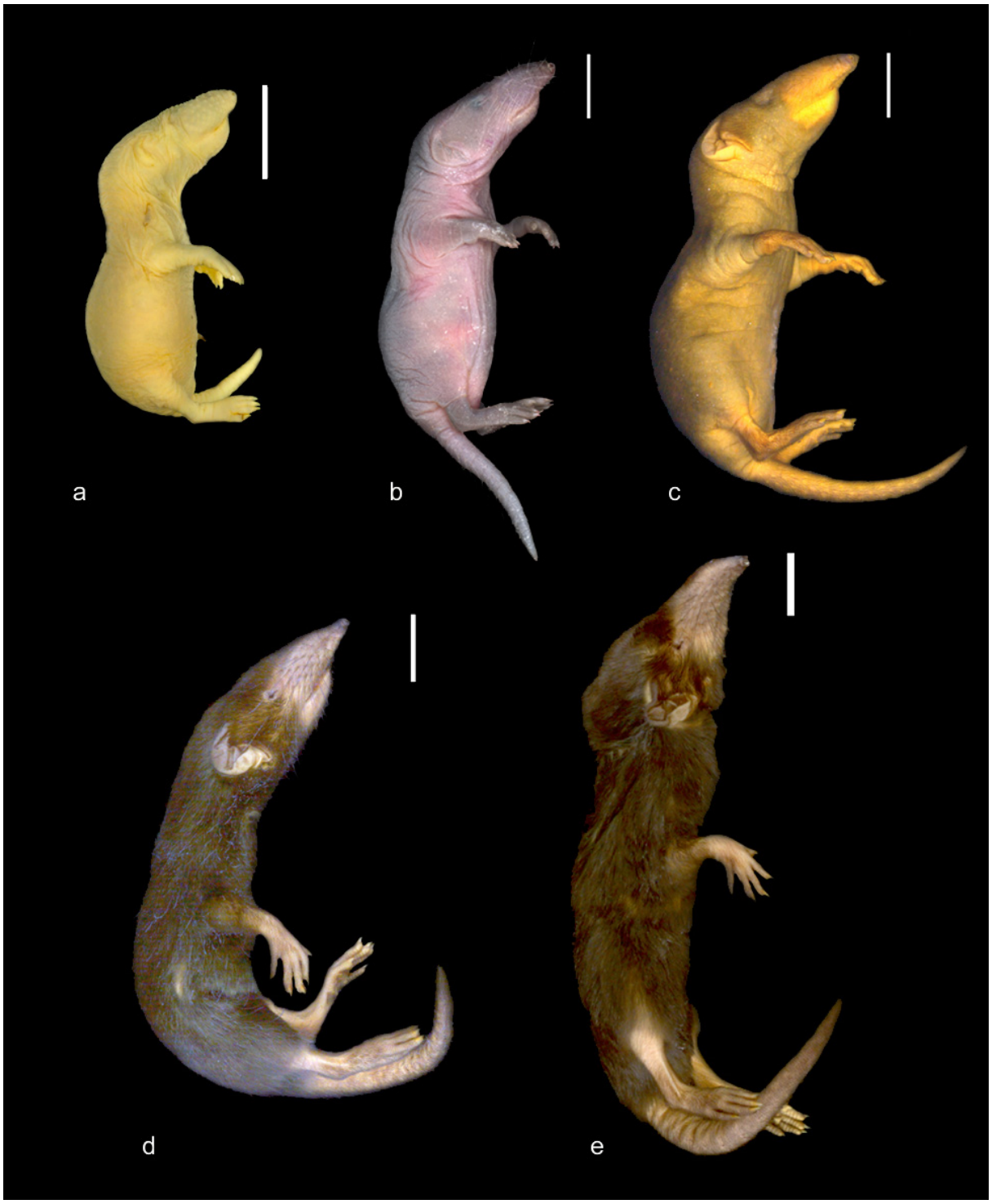

Fig. 16: Postnatal development of *Suncus murinus*. Lateral view of young at day 0 (a), 4 (b), 7 (c), 11 (d) and 14 (e). Scale bar = 1 cm.

incisors takes place. The muscular coordination is well developed and the young are able to coodinate locomotion. Caravan formation similar to that described in other Crocidurinae (Niethammer, 1950; Grünwald and Möhres, 1974) and in *Suncus murinus* (De, 1947; Dryden, 1968) was also observed in our colony. The young attached themselves by mouth to the tail base of the female. This occured usually when the young were disturbed or were

separated from the female before. The direct contact with the female was preferred by the young. At the age of 14 days *Suncus murinus* has a weight of 24.6 g and a CRL of 85 mm (Fig. 16 e). The fur is growing longer and becomes wolly. Apart from that, the external appearence is marked by no clear-cut developmental phenomena. With 14 days the young starts feeding solid food, but is additional nursed for the following week. The onset of weaning is between 17 and 20 days. At the age of 21 days the young is independent. Female *Suncus murinus* become sexually mature at an age of 30 days, males become sexually mature with 50 days (Puschmann, 2004).

3.1.2.5 Tupaia belangeri

The Belanger's tree shrew *Tupaia belangeri* is another altricially born Placentalia. The growth curve shows a zigzag run of the curve, what points out to the fact that young tupaias are nursed once every 48 hours (Fig. 17). The continuous increase of the curve from day 30 can be explained with the abandonment of the nest by the young. The tupaias are feeding now also solid food and the phase of weaning begins.

The morphological development proceeds rapidly in *Tupaia belangeri* (Fig. 18). At the age of four days the young has a weight of 30.2 g and a CRL of 80 mm (Fig. 18 b). The back is pigmented, while the body underside and extremities are pink coloured. Due to the

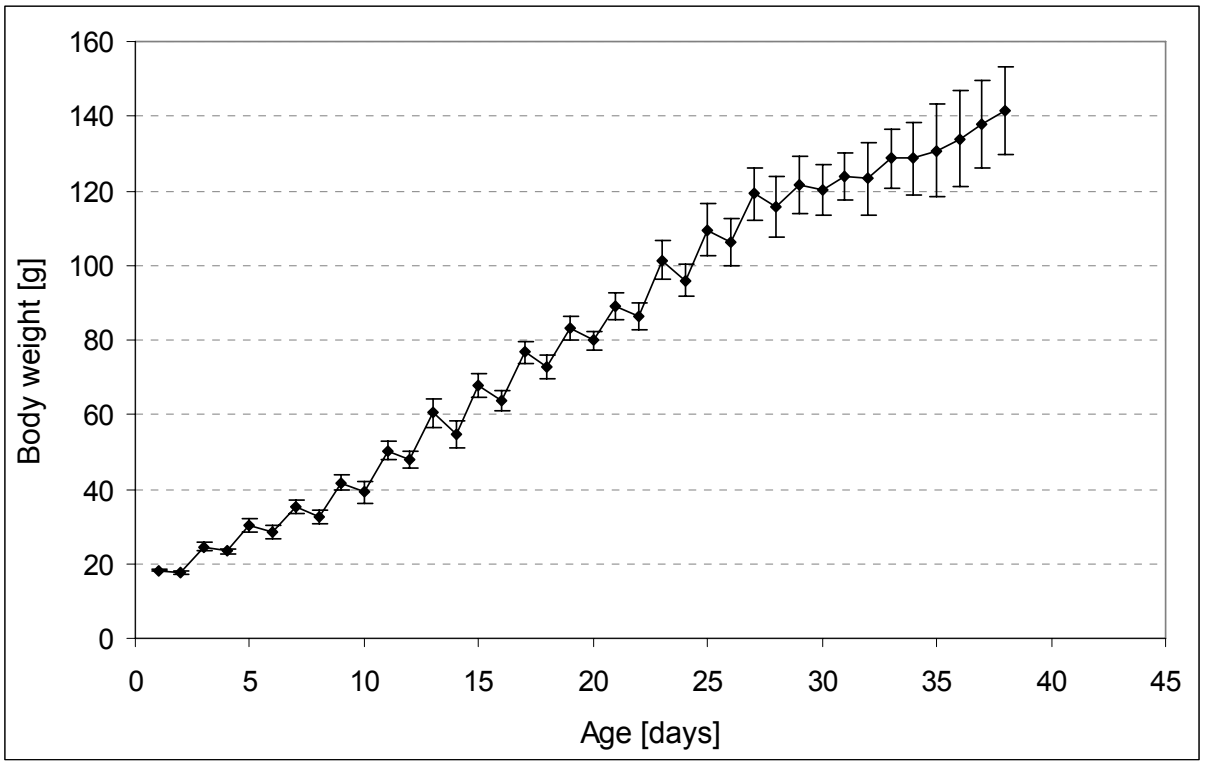

Fig. 17: Growth curve of *Tupaia belangeri* during the first six weeks of life

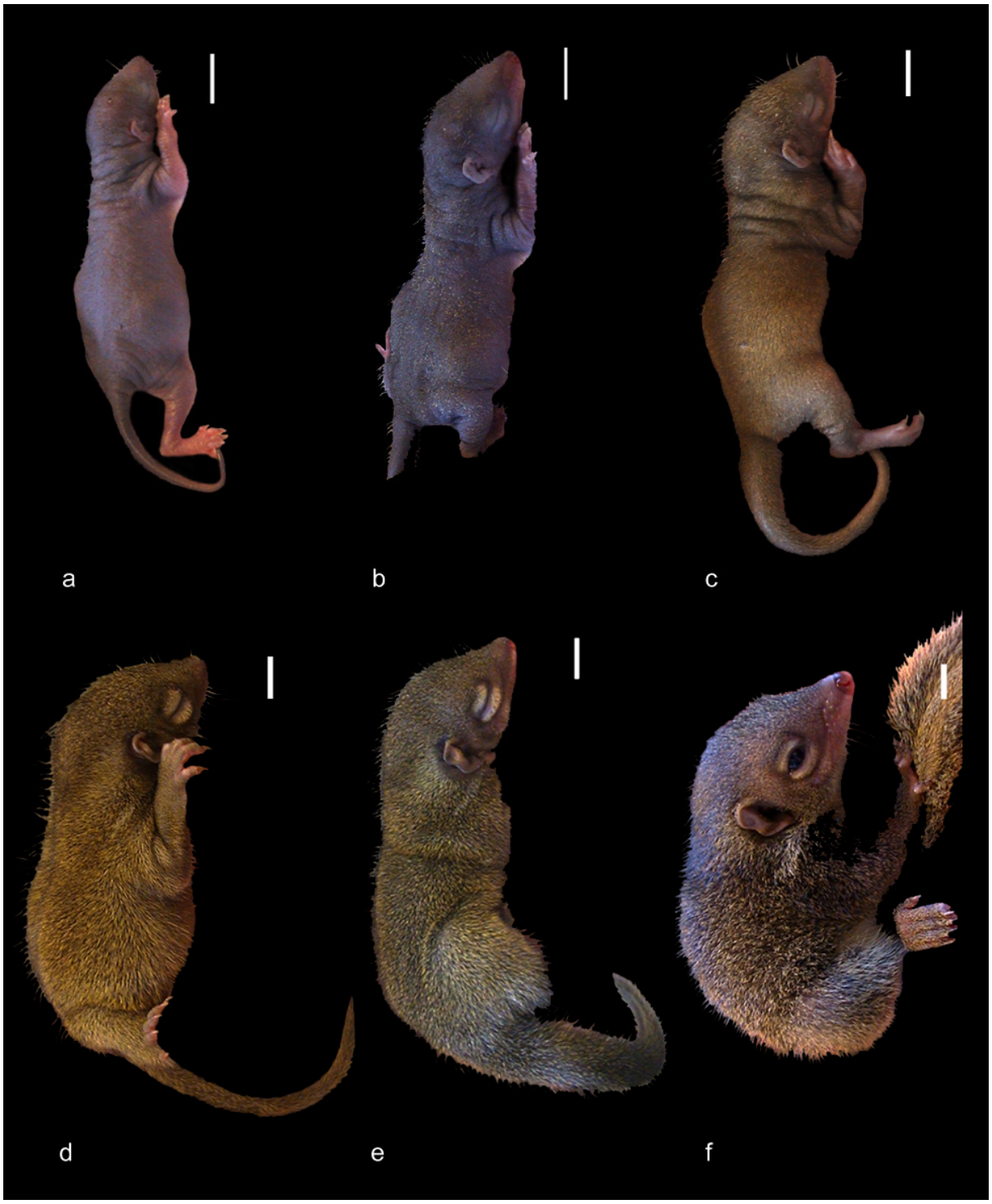

Fig. 18: Postnatal development of *Tupaia belangeri*. Lateral view of young at day 0 (a), 4 (b), 7 (c), 11 (d), 14 (e) and 21 (f). Scale bar = 1 cm.

pigmentation, the young appear grey in colour. Fine hairs are present at the back and upper side of the tail. The eyes and ears are closed and teeths are not present yet. With seven days the young *Tupaia belangeri* has a weight of 32.5 g and a CRL of 85 mm (Fig. 18 c). The fur is clearly visible and shows a green gleam. At the age of 11 days the young has a weight of 47.8 g and a CRL of 92 mm (Fig. 18 d). The fur starts growing at the tail and the underside

of the belly as well as at the forearms and lower legs. The fair shoulder stripes appear at this time. The dental breakthrough begins with the lower incisors. The incisors form the so-called dental comb which is used for combing the fur. With 14 days the young has a weight of 67.9 g and a CRL of 100 mm (Fig. 18 e). The undersides of the tail and the belly are completely furred now. The fur appears more dense and scrubby than that of adults. The eyes and ears are still closed. At the age of 21 days *Tupaia belangeri* has a weight of 86.4 g and a CRL of 120 mm (Fig. 18 f). The eyes open at the age of 19 to 20 days. The process of eye opening can be finished within few hours, but can also last several days. It can proceed in both eyes at the same time or timewise postponed. Normally the eyelids start to separate from the nasal side. The auditory canal is closed up to day 20 by a membrane. After that time the ears are fully functional. In addition to the already present incisors canines and premolars break through. Between 30 and 33 days of life the young is able to coordinate locomotion and leaves the nest for the first time. During the first days after leaving the nest the climbing of the young is unhandy and it often gets unbalanced. During the excursions the young are not guided or protected by the female. From day 30 to 33 *Tupaia belangeri* starts to feed solid food for the first time. After leaving the nest the young is nursed for one week. In contrast to the previous nursing in the nest box the young hassles the female and is nursed on a perch. With 38 days the young is weaned. In *Tupaia belangeri* the females become fertile with three months and the males breed for the first time between four and five months (Fuchs, 1999).

3.1.2.6 Cavia aperea

As a typical representative of precocial placentals *Cavia aperea* is born at a highly advanced developmental stage, as described before in 3.1.1. The newborn *Cavia aperea* has a comparatively high birth weight of 60.5 g. After a short delay in weight gain during the first two days the postnatal growth rate is almost linear and body weight increases steady with about 5.1 g per day (Fig. 19). From the fourth week of life the growth rate decelerates to 3.9 g per day. The morphological development is advanced and many characteristics are already present at birth (Fig. 20). The lateral view at young *Cavia aperea* from newborn to the age of 21 days shows clearly the increase in body length during the postnatal development. Also the shape of the body changes during this time. It alters from the compact newborn form with a relatively round-shaped head to the lengthened adult form with an elongated head. As developmental characteristics the 1 cm.

weaning at the age of 21 days and the initiation of sexual maturity with one to two months in the female and two to three months in the male *Cavia aperea* are to mention (Puschmann, 2004).

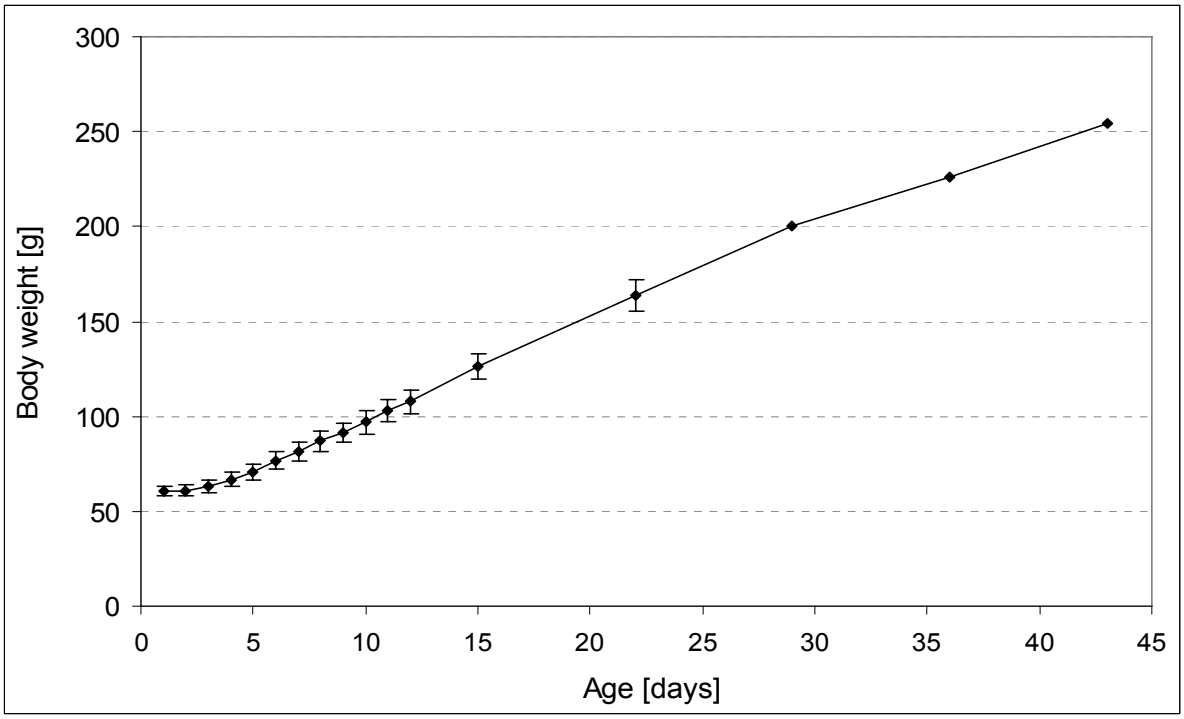

Fig. 19: Growth curve of *Cavia aperea* during the first six weeks of life

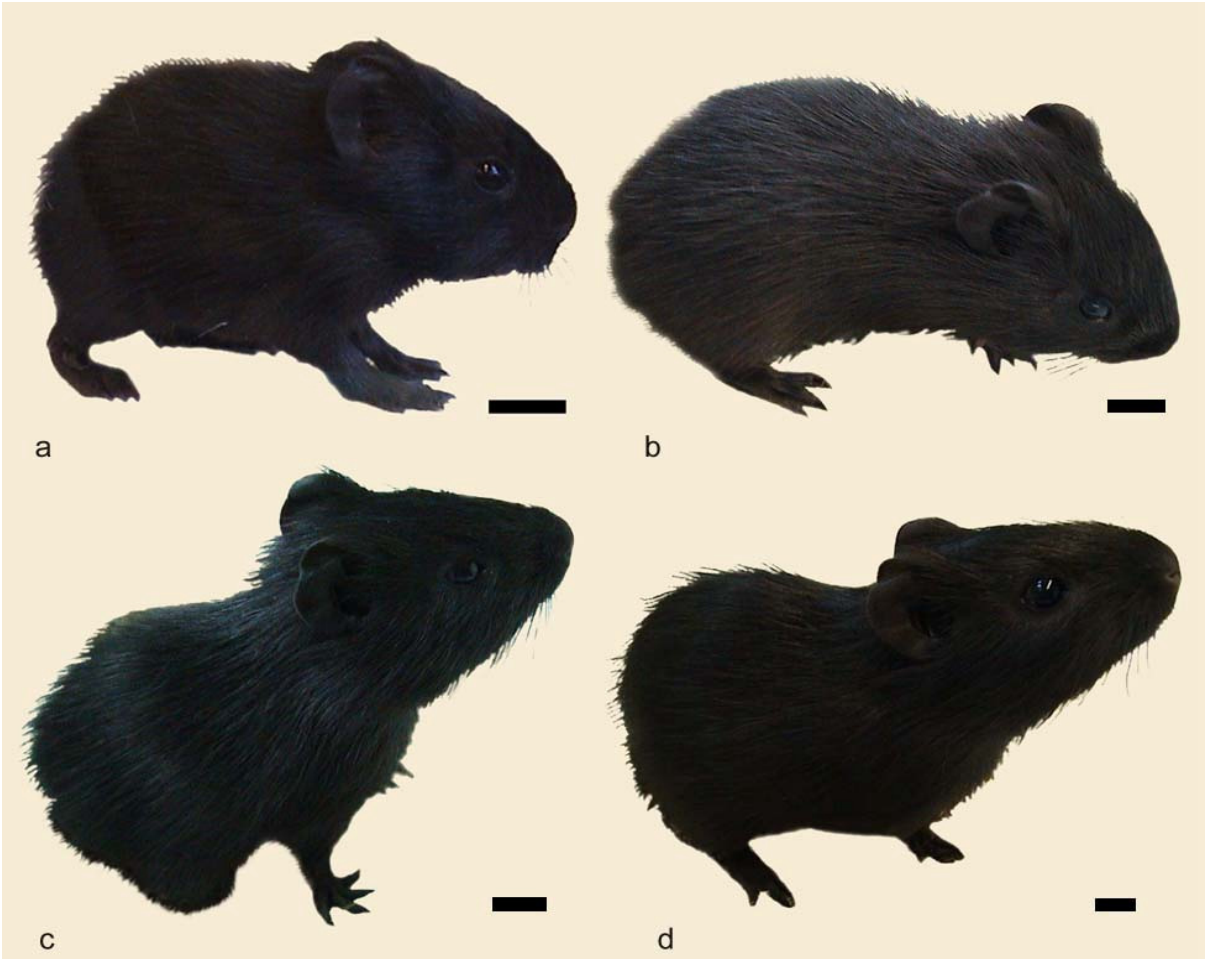

Fig. 20: Postnatal development of *Cavia aperea*. Young at day 0(a), 7(b), 14(c) and 21(d). Scale bar = 1 cm.

3.1.3 Summary of developmental characteristics

The most important characteristics on reproduction and development of the six species examined are summarized in table 2.

Tab. 2: Data on reproduction and development for the four placental and two marsupial species (own observations, supplemented with literature data)

	Placentalia				Marsupialia	
Order	Scandentia	Eulipothyphla	Rodentia		Didelphoidea	Macro-podoidea
Species	*Tupaia belangeri*	*Suncus murinus*	*Mesocricetus auratus*	*Cavia aperea*	*Monodelphis domestica*	*Macropus eugenii*
Female adult weight	151-212 g	38 - 46 g	90 - 110 g	400 - 600 g	80 - 100 g	5000 g
Gestation period	45 days	31 days	16 days	61 days	14 days	29 days
Litter size	2 - 3	1 - 3	6 – 11 (~7)	1 – 3	3 - 14 (~8)	1
Litters / year	7 - 8	2 - 3	up to 8	4 – 5	2	1
Neonatal weight	18.3 g	2.8 g	2.1 g	60.5 g	0.1 g	0.4 g
Age of eye opening	19-20 days	8-9 days	11-13 days	0 days	28 days	140 days
Age of ear opening	20-21 days	5-9 days	4 days	0 days	14 days	>104 days
Age of furring	4 days	4 days	4 days	0 days	28 days	180 days
Attachment	-	-	-	-	14 days	105 days
Permanent pouch exit/ left in nest	-	-	-	-	21 - 28 days	250 days
Weaning	33 – 37 days	17 - 20 days	21 days	21 days	49-56 days	270 days
Sexual maturity	♀ 3 - ♂ 4-5 months	♀ 30 - ♂ 50 days	♀ / ♂ 28-35 days	♀1-2 - ♂2-3 months	♀ / ♂ 120-140 days	♀ 8 - ♂ 24 months
References	Weigold, 1979; Fuchs, 1999	Dryden, 1968; Puschmann, 2004	Whittaker, 1999; Puschmann, 2004	Trillmich, 2000; Puschmann, 2004	Kraus & Fadem, 1987; Hsu, 1999	Tyndale-Biscoe & Renfree,1987

3.2 Histological and ultrastructural findings of lung development

The terms and definitions for the description of the structure and development of the mammalian lung, used in this study, refer to Weibel (1980), Burri (1985) and Netter (1997). With respect to the terminology of the conducting airways of the developing lung a comment is necessary. The term “bronchus” is generally used to name airways with cartilage and seromucous glands in their walls (Weibel, 1980; Burri, 1985; Schiebler and Peiper, 1984). But in contrast to large mammals, in most small mammals the main airways (main, lobar, segmental bronchi) are intrapulmonar without cartilage in their walls. That no cartilage is necessary for stabilisation may be a question of size. For comparative reasons and to distinguish the large airways from the smaller airways (bonchioles) the term “bronchus” is used for these structures although no cartilage is present.

3.2.1 Marsupialia

Marsupials are born after a short gestation period at an early stage of development. The organ systems of the marsupial neonates are generally in an embryonic condition when compared to the placental counterpart (Walker and Gemmell, 1983; Gemmell and Selwood, 1994). Most of their growth and development, including the lung development, occur when they are attached to the teat. For the neonate, the birth is associated with an marked change in the environment. Although the pulmonary maturation proceeds outside the uterus, the newborn marsupial lung has to be already prepared for extrauterine life.

3.2.1.1 Monodelphis domestica

The lung development of the gray short-tailed opossum *Monodelphis domestica* was investigated before by Heiko Schmidt (Schmidt, 1996). The study examined *Monodelphis* as a model of a physiological preterm neonate and compared it to human premature infants. For my comparative approach of different mammalian species more detailed investigations of the lung structure and pulmonary maturation of *Monodelphis domestica* are necessary.

The neonate of Monodelphis domestica is born 14 days after conception and continues to develop attached to the maternal teat, because a marsupium is missing in this species. Lung development during this postnatal period includes both growth and differentiation. A survey of the structural changes during the postnatal lung development is shown in the figures 21.1 and 21.2. At birth, the lung consists of a primitive system of branching airways that end in a

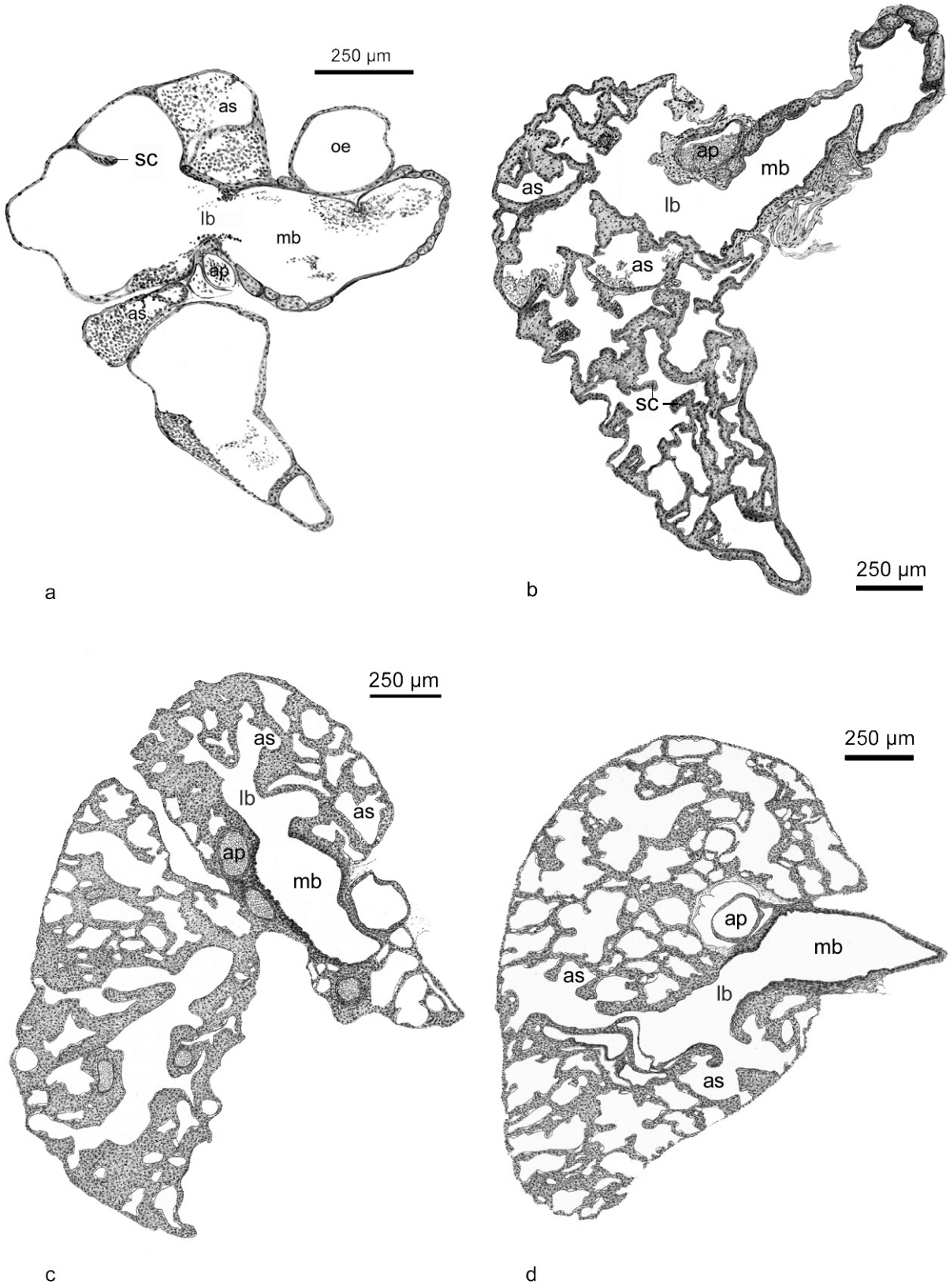

Abb. 21.1: Lung structure of *Monodelphis domestica* during the postnatal development. Original drawings from histological sections of the right lung in a neonate (a) and a 5 (b), 8 (c) and 14 (d) days old *Monodelphis domestica.* ap, pulmonary artery; as, air sac; lb, lobar bronchus; mb, main bronchus; oe, oesophagus; sc, septal crest. Magnification 100 x.

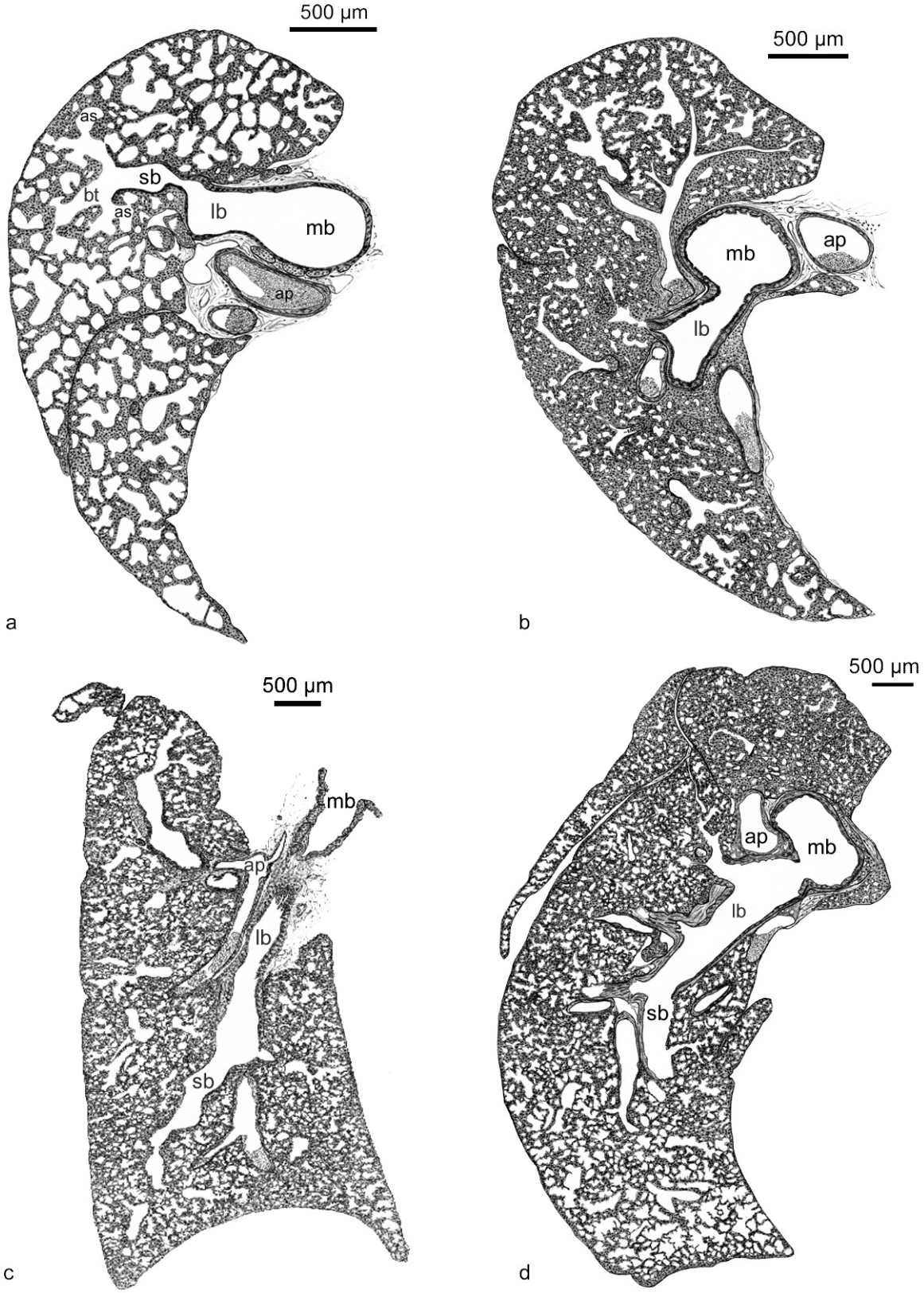

Abb. 21.2: Lung structure of *Monodelphis domestica* during the postnatal development. Original drawings from histological sections of the right lung of a 21 (a), 28 (b), 42 (c) and 56 days (d) old *Monodelphis domestica.* bt, terminal bronchiole; ap, pulmonary artery; as, air sac; lb, lobar bronchus; mb, main bronchus; sb, segmental bronchus. Magnification a, b 50 x; c, d 25 x.

number of large terminal air sacs which are lined with an epithelium that allows respiratory air exchange but which also retains the potential for future growth and differentiation (Fig. 21.1 a). During the early postnatal phase the large air sacs become more and more subdivided by septal crests and decrease in size (Fig. 21.1 b, c). The chambers next to established bronchi differentiate and become incorporated into the bronchial tree. At this time, new air sacs develop at the most distal parts of the bronchial system (Fig. 21.1 d). The air sacs become smaller and more numerous and a ramified bronchial tree is developed (Fig. 21.2 a). The first alveoli can be found at the age of four weeks (Fig. 21.2 b). The following period is characterised by an increase of the number of alveoli. The formation of additional alveoli continues postnatally until adult lung proportions are attained (Fig. 21.2 c, d). The typical lung structure of adults is reached with the formation of alveolar ducts and alveolar sacs (Fig. 21.2 d). The succeeding detailed description of the lung development of *Monodelphis domestica* is subdivided in the postnatal developmental stages examined.

The lung of the neonatal *Monodelphis domestica*

The newborn lung of *Monodelphis domestica* is at the early terminal air sac stage of lung development. At this time the gas exchange takes place at the saccate terminal portions of the airways. These large terminal air sacs have a large lumen of 300 - 400 µm in diameter and are subdivided only a little (Fig. 22 a and 23). They go off directly from the lobar bronchi near the main bronchus.

In general the right lung is slightly larger than the left one. The longitudinal expansion of the right lung is 2.9 mm, compared to 2.8 mm of the left lung. Relating to the CRL of 10 mm, the right lung takes nearly a third of the whole body length. The right lung consists of four pulmonary lobes, the superior lobe, the middle lobe, the inferior lobe and the accessory (cardiac) lobe (Fig. 22 a).

However, the left lung appears to be unlobated and has a single lobe formed by the union of the middle and inferior lobes. The bronchial tree of the right lung divides dichotomic to maximal 22 times and supplies also far distal situated air sacs. On account of the lower size, the left bronchial tree divides less often, maximal 12 times. After the bifurcation of the trachea, the two main or primary bronchi extend to the lower third of the lung. Although several bigger and smaller bronchi branch off from the main bronchus, only a slight constriction of the lumen from 350 µm in the superior part to 250 µm in the inferior part takes place. A schematic reconstruction of the bronchial tree is presented in figure 22 b. The first bronchus which branches off dorsal from the right main bronchus supplies the superior lobe of the right lung.

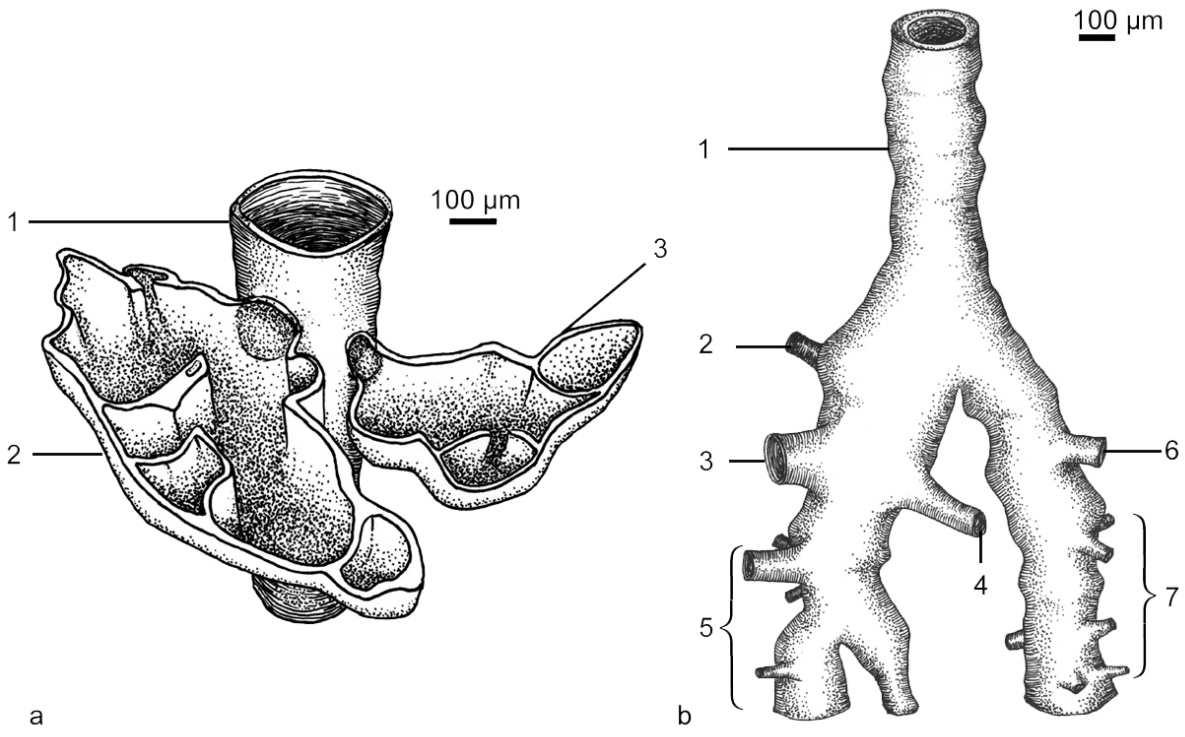

Fig. 22: Reconstruction and schematic representation of the main bronchus of the right lung with bronchi of middle lobe and accessory lobe branching off (a) and of the bronchial tree (b) of the newborn lung of *Monodelphis domestica*. a: 1 – main bronchus; 2 – middle lobe; 3 – accessory lobe. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 – inferior lobe bronchi. Magnification 100 x.

The next is a big bronchus on the lateral side of the main bronchus for the supply of the middle lobe of the right lung. On the same level a small bronchus for the accessory lobe branches off medial from the main bronchus. In the inferior part of the main bronchus several bronchi of different size follow for the supply of the inferior lobe. In the left lung no fissures are present to separate the pulmonary lobes from each other. Indeed, the existence of lobes is indicated by the corresponding bronchi. Lateral from the left main bronchus, the middle lobe bronchus for the supply of the superior and middle part of the left lung branches off. For the supply of the inferior part of the left lung several smaller bronchi branch off at the distal end of the main bronchus. Light microscopic and electron microscopic investigations enable a closer look at the tissue structure and ultrastructural findings of the respiratory airways (Fig. 23 and 24).

The two main bronchial tubes have a diameter of 250-350 µm. They are covered with one-layered cuboidal epithelium. Near the hilum, cartilage is present. The cartilage extends to the second dichotomy along the main bronchial tubes. The most lobar bronchi, that branch off from the main bronchi, are very short and end directly in the capillarised large air sacs (Fig. 23 a). Only the tracheas as well as the upper two thirds of the main bronchus and some

proximal bronchi are solely conductive. The distal parts of the air sacs have only respiratory function. But the most bronchi and the proximal parts of the long air sacs have conductive as

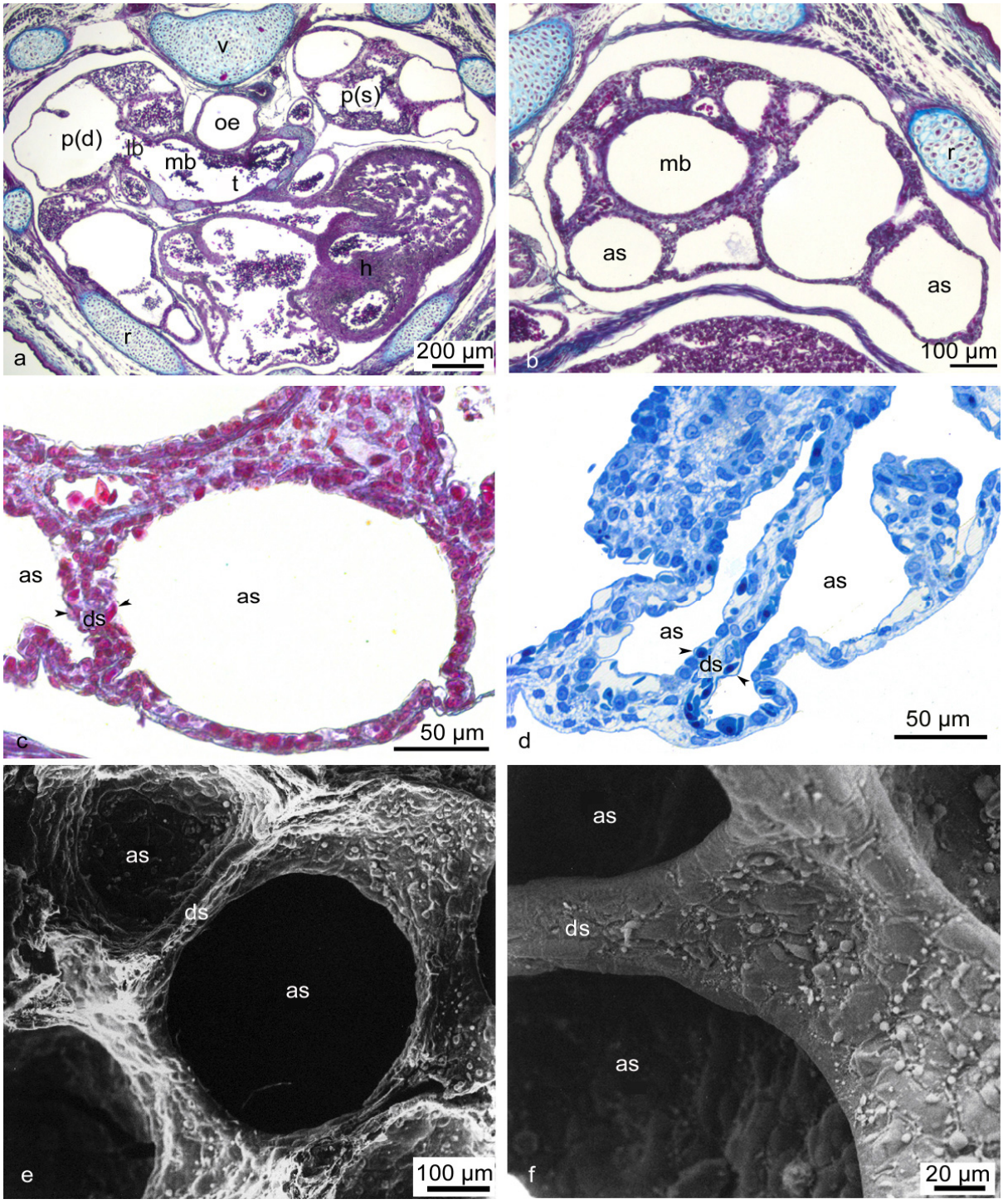

Fig. 23: Light micrographs (a-d) and scanning electron micrographs (e, f) of the newborn lung of *Monodelphis domestica*. The lung is at the early terminal sac stage and consists of large terminal air sacs of 300-400 µm in diameter (b). The large air sacs branch off directly from the main bronchus or from short afferent bronchi (a). The air sacs are separated by a thick septum (e, f). The septum consists of a double capillary bed (c, d; arrows). as, air sac; h, heart; lb, lobar bronchus; mb, main bronchus; oe, oesophagus; p(d), right lung (pulmo dextra); p(s), left lung (pulmo sinistra); ds, double capillary septum; r, rib; t, trachea; v, vertebra. Azan staining. Magnification is indicated by the scale bar.

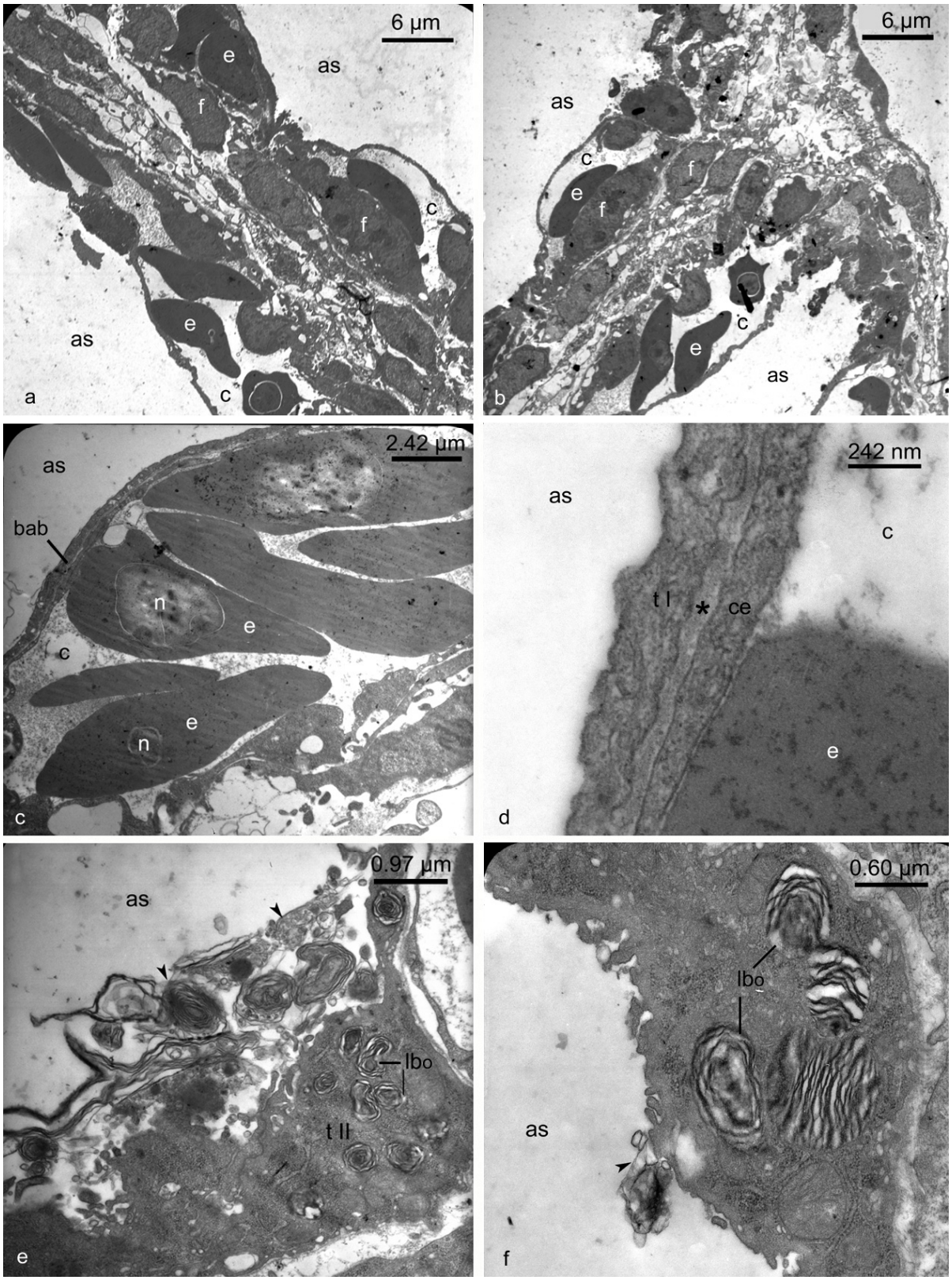

Fig. 24: Transmission electron micrographs of the newborn lung of *Monodelphis domestica*. In the neonatal lung an extensive double capillary network with a mass of mesenchyme is present (a, b). The erythrocytes are numerous but still nucleate (c). The blood-air barrier shows a trilaminar structure of type I pneumocytes, endothelial cells and a fused basal lamina of both cell types (∗) (d). The cuboidal type II pneumocytes are common. They produce a large quantity of phospholipids inside the lamellar bodies (e, f) and secrete it in the form of surfactant (arrows) (f). as, air sac; bab, blood-air barrier; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

well as respiratory function. The large terminal air sacs have a diameter of 300-400 µm (Fig. 23 b, c, e). Many air sacs have small bulges of 50-100 µm, which indicate a further proliferation process. The air sacs are lined with a simple squamous sheet of epithelium that lies on top of an extensive capillary bed. Between the simple squamous type I pneumocytes cuboidal type II pneumocytes are interspersed (Fig. 24 e). The type II pneumocytes carry short microvilli in their apical surface. In the cytoplasm of these cells Golgi apparatus, rough endoplasmic reticulum, fat vacuoles and numerous osmiophilic lamellar bodies are present. The lamellar bodies are surrounded by a membrane within the type II pneumocytes. These multilamellar corpuscles are an important component of the type II pneumocytes, because they are the place of surfactant synthesis (Fig. 24 f). They are found as whole corpuscles, as well as single lamella fragments. After synthesis and maturation in the lamellar bodies the surfactant is secreted by the type II pneumocytes and covers in a variable thick layer the surface of the air sacs. Surfactant consists mainly of phospholipids and is separated by a hypophase from the surface of the air sacs. Its function is to decrease the surface tension of the air sac surface so that the diameter of the air sac is stabilised.

Each terminal air sac has its own capillary bed that is separated from the capillary bed of the adjacent air sac (Fig. 23 d, f). Thus an extensive double capillary network exists in this developmental phase. Beside isolated thick isles of connective tissue, the septa located between the air sacs have an average thickness of about 30 µm (Fig. 24 a, b). The interstitial layer of the double capillary septum contains delicate reticular fibers and mesenchymal cells. The septum may contain also larger capillaries. The pulmonary vasculature is well developed with short blood-air diffusion distances. The blood-air barrier between the capillaries and the air sac lumen has a thickness of 0.5-1 µm and contains capillary endothelial cells, type I pneumocytes, and a fused basal lamina of both cell types (Fig. 24 d). The erythrocytes in the capillaries are still nucleate to the date of birth, so that the capacity of oxygen transport in the blood is low (Fig. 24 c).

The lung of the five days old *Monodelphis domestica*

The lung of a five days old *Monodelphis domestica* distinguishes from that of a newborn particularly through an increasing subdivision of the terminal air sacs (Fig. 25 a). The histological and ultrastructural findings of the lung structure of the five days old *Monodelphis* are presented in the figures 25 and 26.

The bronchial tree corresponds to that of a newborn Monodelphis domestica; however, the main bronchi increase in length. The lobar bronchi branching off from the main bronchus have thin respiratory epithelium and with that also respiratory function. The solely conducting portion of the airways is very small and is restricted to the proximal parts of the main and

lobar bronchi. The terminal air sacs branch off directly from the short afferent lobar bronchi (Fig. 25 b, e).

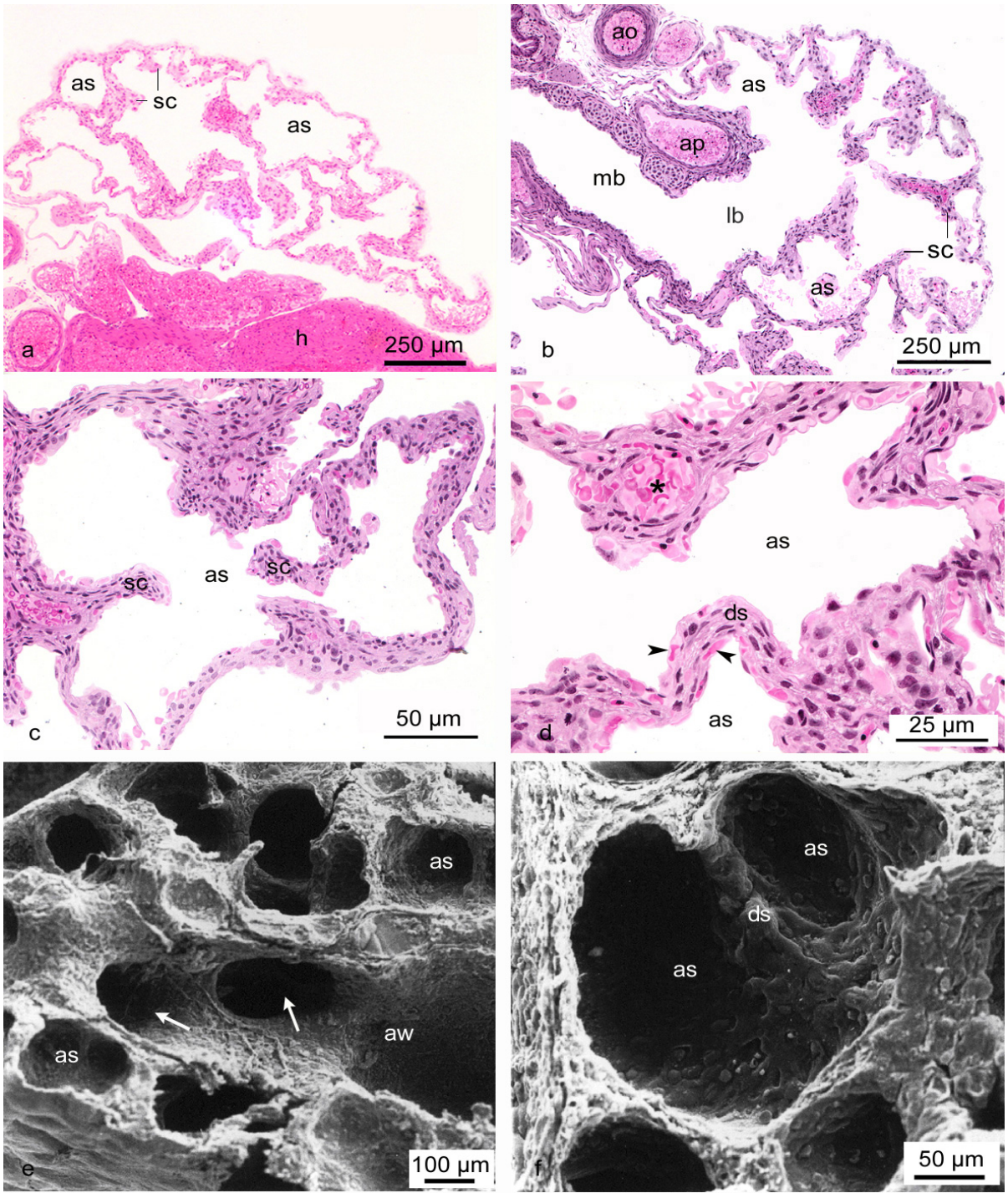

Fig. 25: Light micrographs (a-d) and scanning electron micrographs (e, f) of the five day old lung of *Monodelphis domestica*. The lung is still at the terminal air sac stage with large terminal air sacs of 200-250 µm in diameter (c, f). The air sacs branch off directly from the main bronchus (b) or from short afferent bronchi (e; arrows indicate the branching of air sacs). Thick septa (d, f) consisting of a double capillary network (d; arrows) separate adjacent air sacs. Thick septal junctions often enclose large blood vessels (d; asterisk). as, air sac; ap, pulmonary artery; aw, airway; h, heart; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sc, septal crest. HE staining. Magnification is indicated by the scale bar.

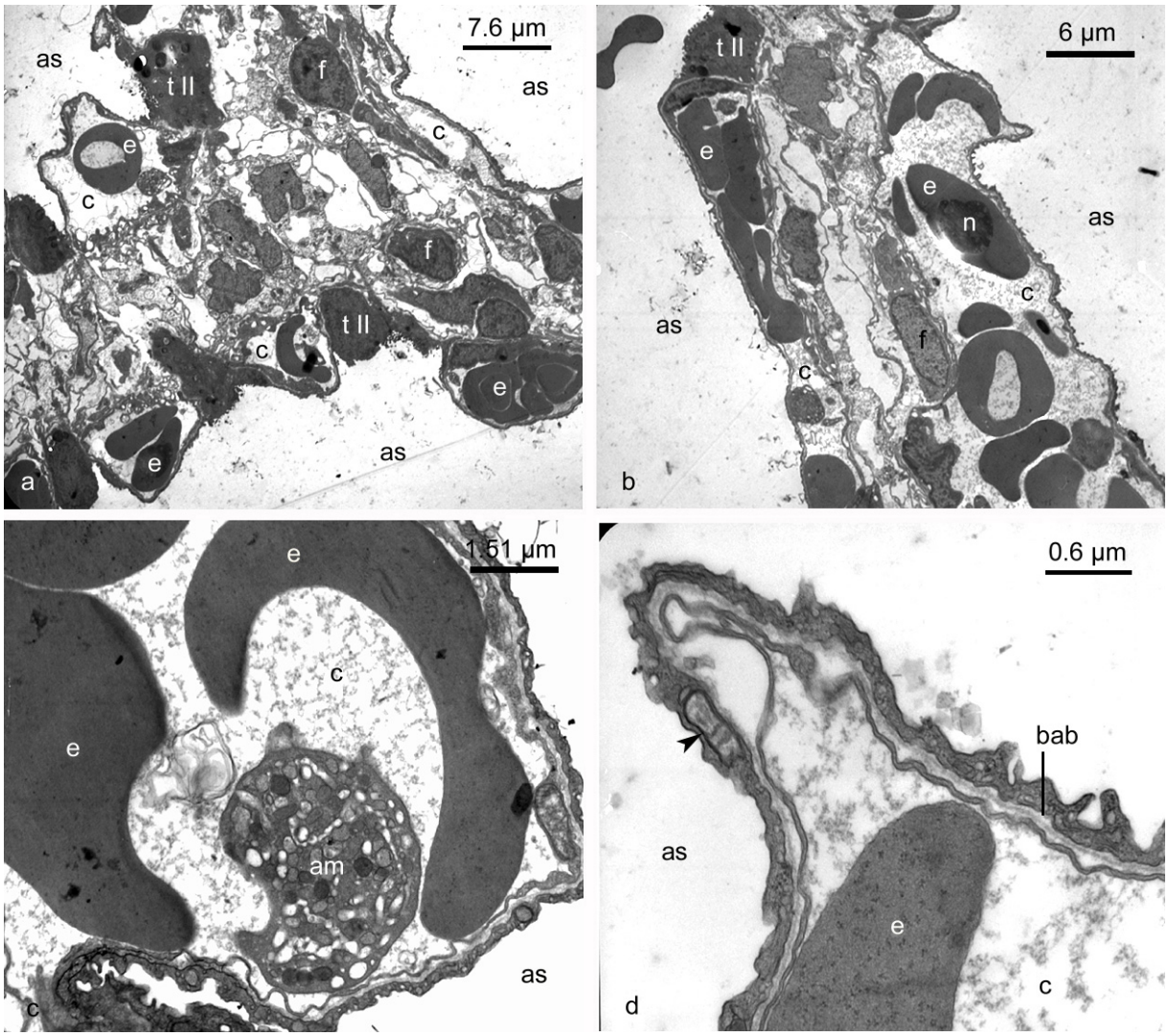

Fig. 26: Transmission electron micrographs of the 5 day old lung of *Monodelphis domestica*. At this stage a double capillary network with an extensive tissue core with large interstitial spaces is present (a, b). The majority of erythrocytes are without a nucleus, but few are still nucleate (b, c). The blood-air barrier with the typical trilaminar structure of type I pneumocytes, endothelial cells and fused basal laminas of both cell types (d) is thinner than in the newborn *Monodelphis.* The type I pneumocytes contain coated vesicles (arrows). The cuboidal type II pneumocytes are present as „niche cells" in the corners of the septa (a). as, air sac; am, alveolar macrophage; bab, blood-air barrier; c, capillary; e, erythrocyte; f, fibroblast; n, nucleus; t II, type II pneumocyte. Magnification is indicated by the scale bar.

The terminal air sacs are large with a diameter of 200-250 µm (Fig. 25 c, f). The surface of the air sacs appears to be smooth-walled. Characteristic for this developmental stage is an amount of septal ridges or developing crests protruding from the septa (Fig. 25 b, c). That indicates a further process of subdivision of the lung parenchyma. The septa forming the walls of the terminal air sacs vary in thickness. Those in the central part of the lung are thicker (30 µm) than the more peripherally located septa (20 µm). The tissue core in the thick septa is composed of stromal cells with thin extensive processes surrounded by large interstitial spaces (Fig. 26 a). Fine collagen strands are scattered in the interstice and fibroblasts are located within the septa. Large blood vessels are commonly found at thick

septal junctions (Fig. 25 d). The thinner peripheral septa have a more compact appearance, with a marked reduction in the amount of interstitial space (Fig. 26 b). Toward the air space the terminal air sacs are lined mainly with squamous type I pneumocytes and occassionally interspersed cuboidal type II pneumocytes. Compared to the lung of the newborn *Monodelphis domestica* the type II pneumocytes seem to be reduced in number. In the newborn lung capillaries and type II pneumocytes frequently alternate. However, the type II pneumocytes in the five days old *Monodelphis* lung are mainly present in the corners of two adjacent septa (Fig. 26 a). The intracellular composition of the type II pneumocytes resembles that of the newborn lung.

As in the neonate a double capillary network is present in the five days old *Monodelphis domestica* (Fig. 26 a, b). Each terminal sac has its own capillary bed. The capillaries are long and they are located at both sides of the septa. In the width they measure 7-8 µm, whereas their length is 30-40 µm. In part the capillaries bulge into the lumen of the air sacs and enlarge the surface of the gas exchange area. The majority of the erythrocytes in the capillaries are already without a nucleus (Fig. 26 c). However, some few are still nucleate (Fig. 26 b). The blood-air barrier between the capillary and the air sac lumen has the same structure as described before in the neonatal *Monodelphis domestica* (Fig. 26 d). However, the blood-air barrier is with a thickness of 300 nm thinner than in the newborn lung. Some type I pneumocytes contain vesicles of various sizes, some are coated (Fig. 26 d, arrow). These vesicles are involved in the transport of proteins.

The lung of the eight days old *Monodelphis domestica*

The main characteristics of the eight days old *Monodelphis* lung are an increasing subdivision of the lung and a gradually decrease in size of the air sacs. A bronchial tree with solely conducting function begins to develop. The histological and scanning electron microscopic findings of the eight days old lung of *Monodelphis domestica* are presented in figure 27. The longitudinal expansions of the right and of the left lung are 3.1 mm and 2.6 mm. With a CRL of 13 mm the right lung takes a fourth of the whole body length.

The bronchial tree develops and the number of dichotomies increases. The terminal air sacs are in connection with the short afferent segmental bronchi, which branch off from the lobar bronchi (Fig. 27 a, b, f). The main bronchi measure 200-250 µm in diameter and are lined with one-layered cuboidal epithelium; in the proximal part cartilage is present. In the distal part the main bronchi are lined with respiratory epithelium. Bronchi in proximity to air sacs show an extensive capillary bed directly subjacent to the epithelium and for this reason they have respiratory function (Fig. 27 b). The distal parts of developing terminal bronchioles

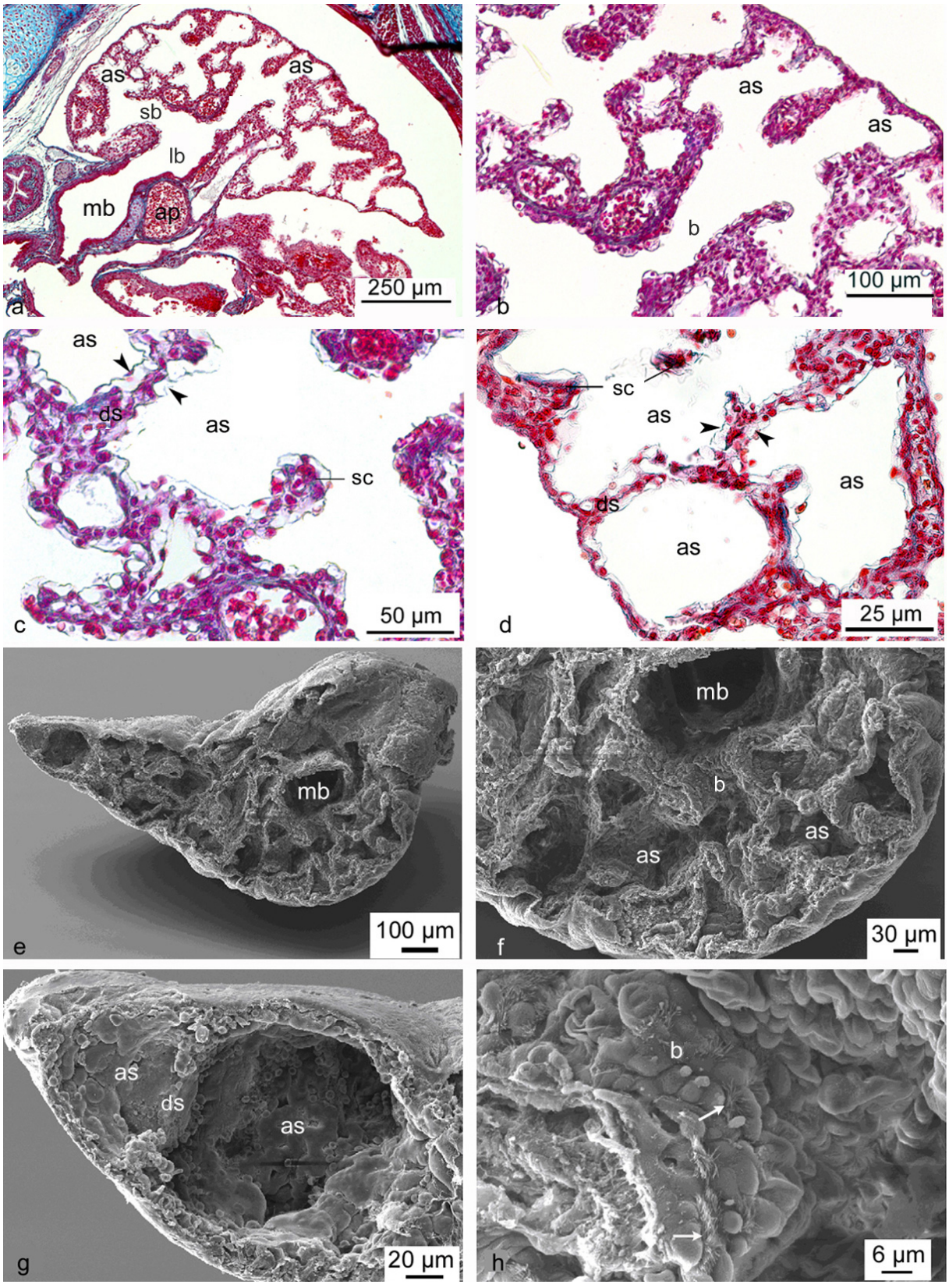

Fig. 27: Light micrographs (a-d) and scanning electron micrographs (e-h) of the eight days old lung of *Monodelphis domestica*. The general view of the lung reveals an increase of dichotomies and smaller air sacs (a, e). The terminal air sacs have a diameter of 150-200 µm (c, d, g). They are separated by a septum with a double capillary bed (c, d; arrows). The air sacs branch off from short bronchioles (b, f). The terminal bronchioles show ciliated cells (h, arrows). as, air sac; ap, pulmonary artery; b, bronchiole; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sc, septal crest. Azan staining. Magnification is indicated by the scale bar.

are lined by a cuboidal epithelium with few scattered ciliated cells (Fig. 27 h). The function of the ciliated cells is to move secretions and trapped airborne particles towards the pharynx.

The terminal air sacs continue to make up a major portion of the lung (Fig. 27 a, e). They have a size of 150-200 µm in diameter (Fig. 27 c, d, g). The smaller terminal air sacs are smooth walled, whereas crests that vary in height and thickness are numerous in the larger air sacs. They give them an irregular shape. The terminal air sacs are lined by the two previously described epithelial cell types, type I and type II pneumocytes. The double capillary septa between the air sacs are still relatively thick. Large blood vessels are located at the septal junctions (Fig. 27 b, c, d).

The lung of the 14 days old *Monodelphis domestica*

The lung of the 14 days old *Monodelphis domestica* is characterised by an increase in the number of smaller terminal air sacs and the continued development of the bronchial tree (Fig. 28 a). The light and scanning electron microscopic findings of the lung structure of a 14 days old *Monodelphis domestica* are presented in figure 29. For the ultrastructure, transmission electron microscopic investigations were carried out[1] (Fig. 30).

With a total length of 6.0 mm, the right lung is slightly larger than the left lung, which measures 5.6 mm in length. Relating to the CRL of 20 mm the right lung takes a third of the whole body length. Compared to the eight days old *Monodelphis* lung a doubling of the lung size took place.

A schematic reconstruction of the bronchial tree of a 14 days old *Monodelphis* is given in figure 28 b. The branching pattern of the lobar bronchi is equivalent to the newborn lung. The schematic representation of the main bronchus of the right lung with middle lobe and accessory lobe bronchi branching off reveals a more differentiated lung structure (Fig. 28 a). The main bronchi measure 250-300 µm in diameter and their structure resembles that of the newborn lung. In addition to the main and lobar bronchi also segmental bronchi and short terminal bronchioles are present. At the distal parts of the terminal bronchioles cuboidal epithelium with ciliated cells can be found (Fig. 29 h). While this cell type was found scattered in the eight days old *Monodelphis* lung, at the age of 14 days a dense covering with ciliated cells is recognisable. The lobar bronchi branching off from the proximal part of the main bronchus are lined solely with conductive epithelium. First after a renewed

[1] In order to reduce the number of animals for the histological and ultrastructural investigations, already present transmission electron microscopic material of a 12 day old *Monodelphis domestica* was investigated for this developmental stage. The lung structure of the 12 day old young is equivalent to that of the 14 days old young.

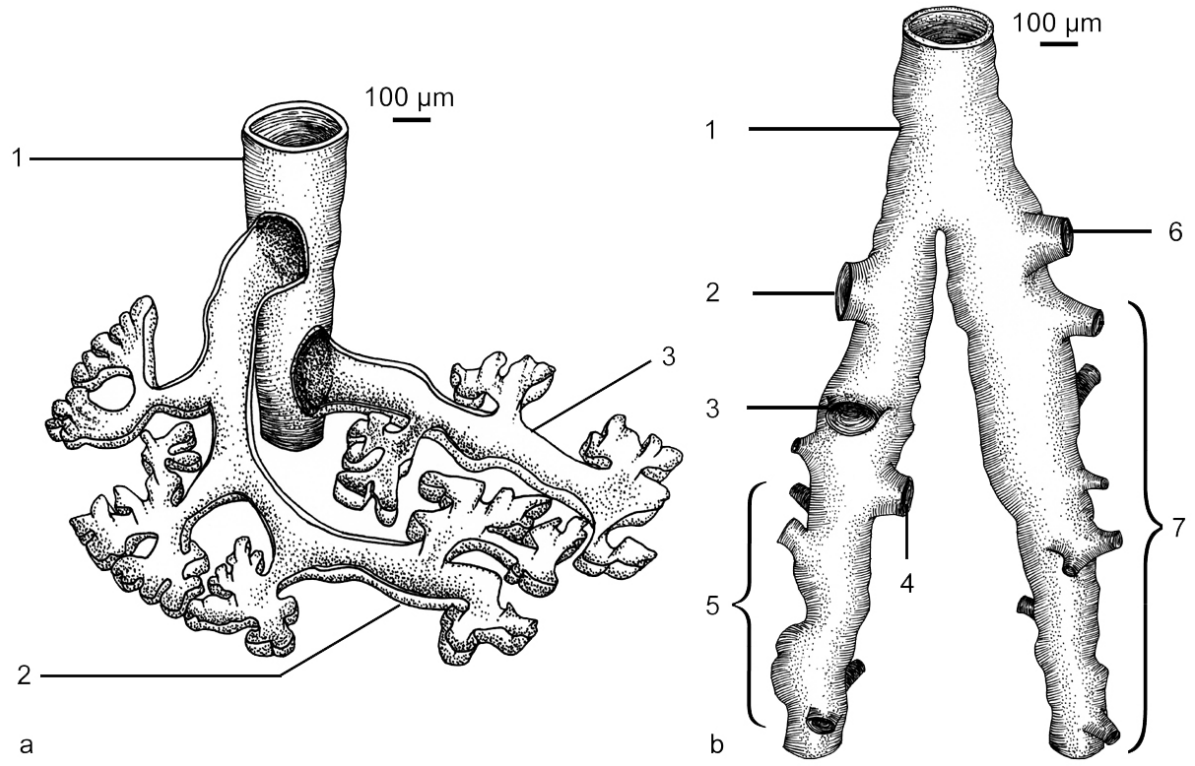

Fig. 28: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the 14 days old lung of *Monodelphis domestica*.a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 - inferior lobe bronchus. Magnification100x.

dichotomy it is replaced by respiratory epithelium. A typical course of a lobar bronchus is a convex curve to the ventral side. On the convex side of the bronchus, numerous segmental bronchi branch off. From that short terminal bronchioles open immediately into air sacs (see Fig. 28 a, bronchus of medial lobe). Besides the solely conductive epithelium of the proximal airways, the peripheral bronchi show still much respiratory epithelium with capillaries at the walls.

The terminal air sacs are more numerous and decrease in size. They measure 100 µm in diameter. Several air sacs develop near the pleura. They are separated by septa vertically standing on the pleura. The air sacs appear smooth walled and they are lined by type I and type II pneumocytes, similar to the previously described developmental stages. The type II pneumocytes are usually found singly or in pairs and they contain prominent lamellar bodies (Fig. 30 c). The air sacs are still separated by a double capillary septum (Fig. 30 a, b). The septa are with 20-25 µm relatively thick, however, the width of the capillaries remains unchanged with 7-8 µm. Similar to the previously described septa, there is a core of stromal cells with thin extensions and large interstitial spaces. The erythrocytes within the capillaries are no longer nucleated at this age. The diffusion barrier is formed by tissue at the septal

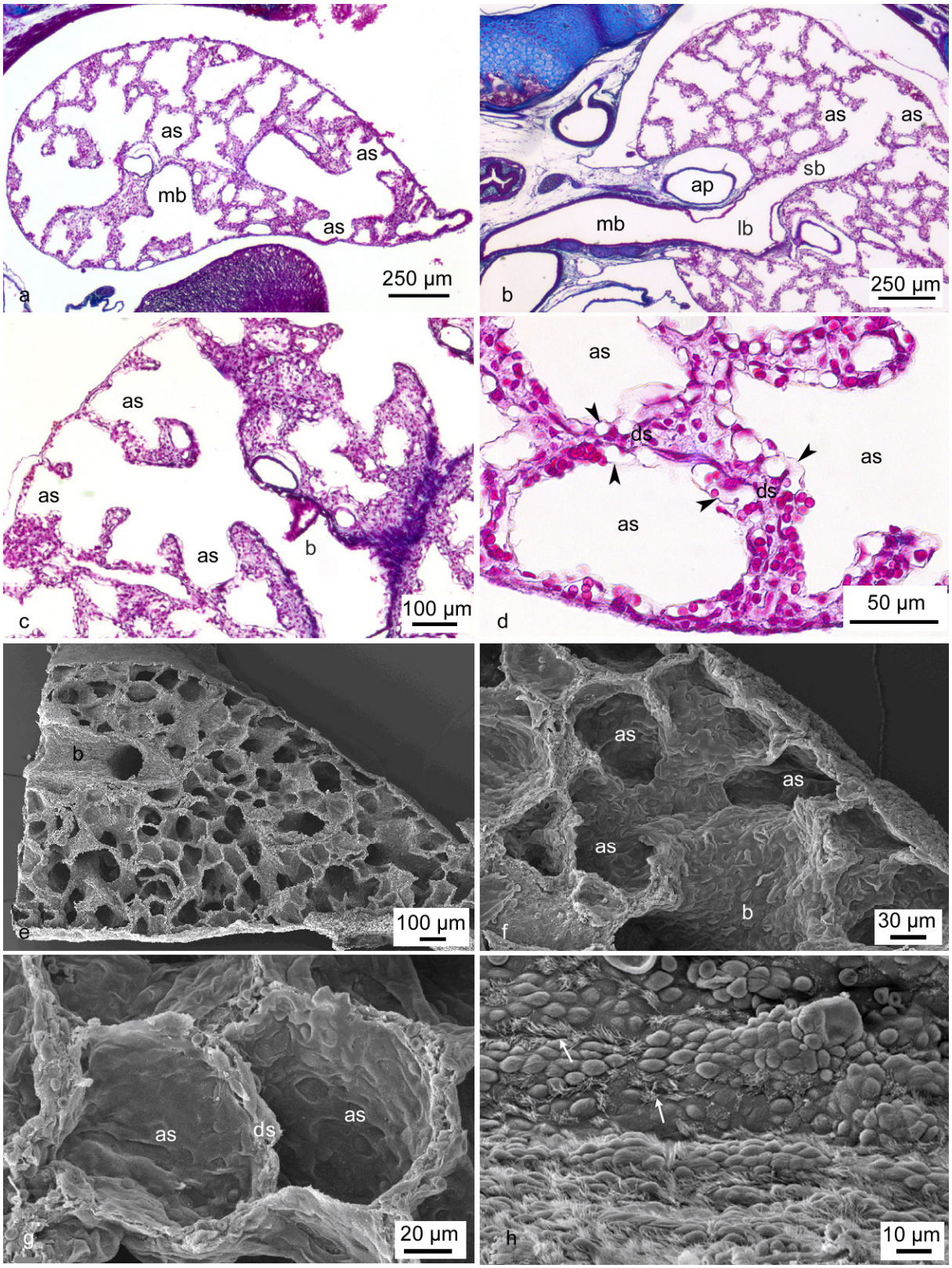

Fig. 29: Light micrographs (a-d) and scanning electron micrographs (e-h) of the 14 days old lung of *Monodelphis domestica*. The main features of the lung are the elongation of the bronchi and the smaller and more numerous air sacs (a, e). The terminal air sacs have a diameter of 100 µm (c, d, g). They are still separated by a septum with a double capillary network (d, g; arrows). The air sacs open from short terminal bronchioles, which branch off from long afferent segmental bronchi (b, f). The terminal bronchioles are densely covered with ciliated cells (h, arrows). as, air sac; ap, pulmonary artery; b, bronchiole; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sb, segmental bronchus. Azan staining. Magnification is indicated by the scale bar.

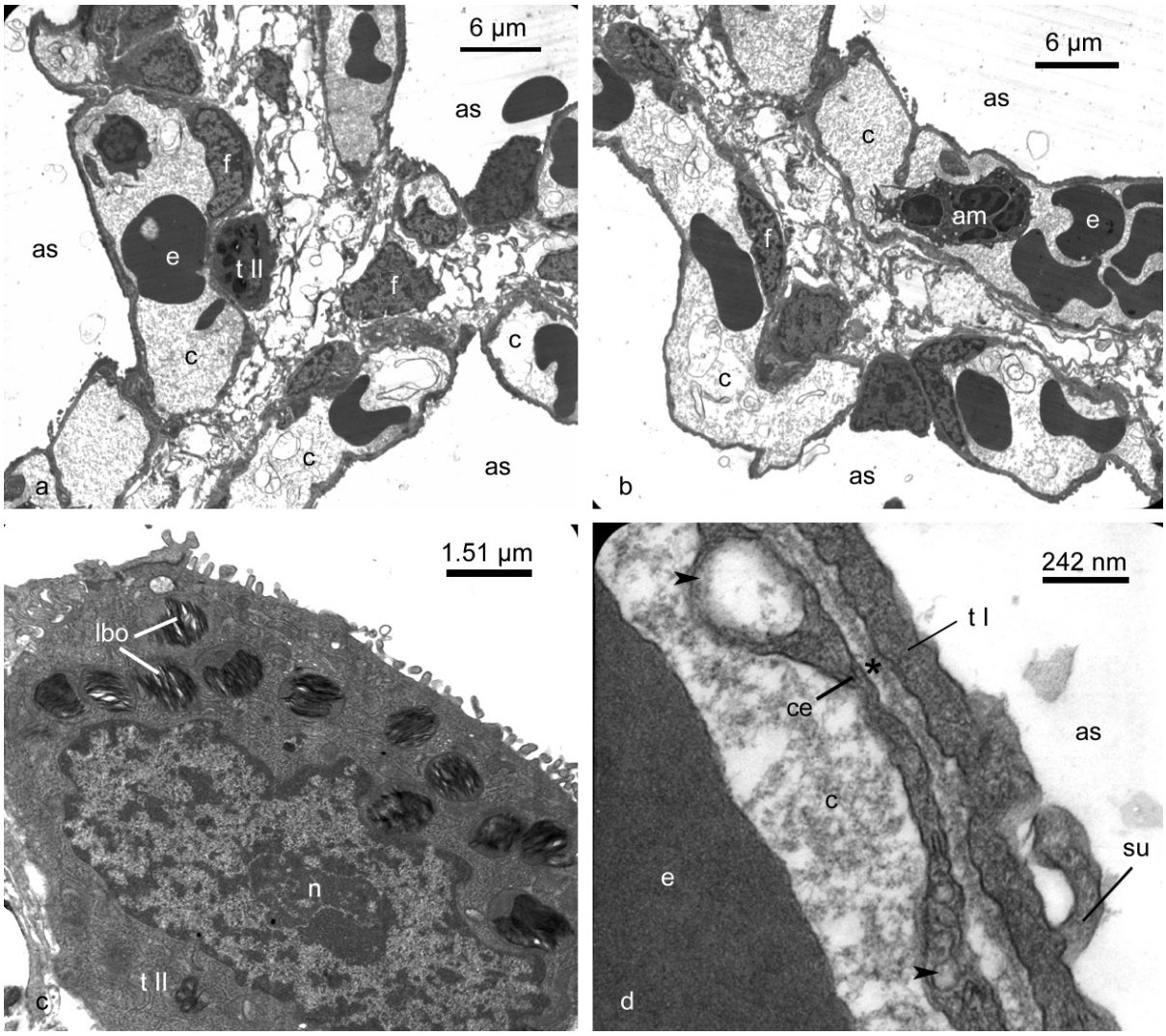

Fig. 30: Transmission electron micrographs of the 12 day old lung of *Monodelphis domestica*. The air sacs are still separated by a septum, composed of a double capillary network (a, b). The blood-air barrier shows the typical trilaminar structure of type I pneumocytes, endothelial cells and a fused basal lamina of both cell types (∗) (d) and towards the air space a thin layer of surfactant. The capillary endothelium contains coated vesicles (arrows). The cuboidal type II pneumocytes are characterised by a considerable quantity of lamellar bodies (c). as, air sac; am, alveolar macrophage; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; t I, type I pneumocyte; t II, type II pneumocyte; su, surfactant. Magnification is indicated by the scale bar.

surface between the air in the terminal air sacs and the blood in the capillaries. It consists of epithelial cells and endothelial cells, separated from each other by a single basal lamina. Apart from the trilaminar structure, the blood-air-barrier becomes progressively thinner with age and measures 250 nm in thickness (Fig. 30 d).

The lung of the 21 days old *Monodelphis domestica*

At the age of 21 days, the lung shows a considerable increase in complexity of the conducting portion of the lung. In addition an increase in the number and a decrease in size of the terminal air sacs took place. The light and electron microscopic findings of the lung

structure of a 21 days old *Monodelphis domestica* are shown in figure 31.

The main bronchi have a diameter of 250-300 µm and are lined with one-layered cuboidal epithelium. The surrounding stroma contains smooth muscle cells. At the proximal part of the main bronchi cartilage is present. Numerous segmental bronchi have arisen additionally to

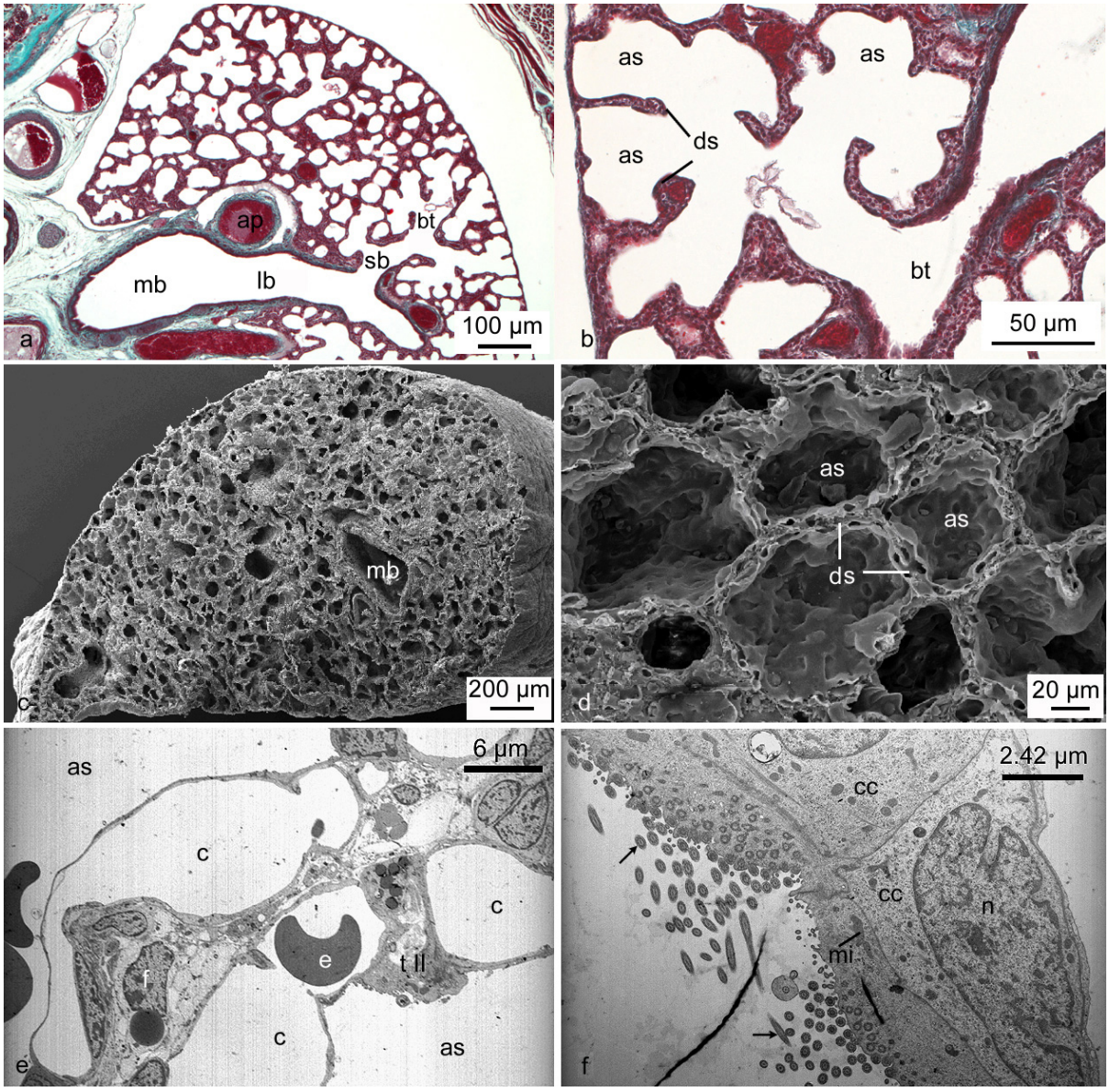

Fig. 31: Light (a, b), scanning electron (c, d) and transmission electron (e-h) micrographs of the 21 days old lung of *Monodelphis domestica*. The bronchial tree appears more differentiated and the air sacs are smaller and more numerous (a, c). The terminal air sacs have a diameter of 50-100 µm and are still separated by a thin double capillary septum (b, d, e). The terminal bronchioles are lined with ciliated cells (g, cilia are indicated by arrows). Type II pneumocytes (f) and the blood-air barrier (h; coated vesicles indicated by arrows; basal lamina indicated by star) are similar in structure as in earlier stages. as, air sac; ap, pulmonary artery; bt, terminal bronchiole; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; mb, main bronchus; mi, mitochondria; n, nucleus; ds, double capillary septum; sb, segmental bronchus; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Trichrome staining. Magnification is indicated by the scale bar.

the main and lobar bronchi. Therefrom various small terminal bronchioles lead to the terminal air sacs (Fig. 31 a, b). Whereas main and lobar bronchi have solely conductive epithelium, segmental bronchi and terminal bronchioles are partially lined with respiratory epithelium. The terminal bronchioles are characterised by one-layered cuboidal epithelium and a thin circular layer of smooth muscle cells. The cuboidal epithelium of the distal part of the terminal bronchioles is ciliated. The ciliated cells have an apical region packed with large mitochondria and cilia at the surface and a basal region containing the nucleus (Fig. 31 f).

The terminal air sacs are with a diameter of 50-70 µm smaller in size and more numerous than in earlier developmental stages (Fig. 31 c, d). The septa between the air sacs are still septa with a double capillary network (Fig. 31 e). The thickness of the septum is with 20-25 µm the same as in the 14 days old *Monodelphis domestica*. However, the capillaries increase in thickness to 12 µm and occupy a bigger part of the septum. In contrast to earlier stages a thick core of stromal cells with large interstitial spaces is missing. The blood-air barrier is in structure and thickness similar to that described in the 14 days old *Monodelphis domestica.*

The lung of the 28 days old *Monodelphis domestica*

The main feature of the lung of the 28 days old *Monodelphis domestica* is the beginning formation of alveoli (Fig. 32 a). The histological and ultrastructural findings of the lung structure of a 28 days old *Monodelphis domestica* are presented in the figures 33 and 34. A first look at the lung reveals a more structured appearence (Fig. 33 a, b, d). The total length of the right lung is 6.3 mm, whereas the left lung measures 6.1 mm in length. Compared to the 14 days stage, only a slight increase in length took place. However, in relation to the total body length of 35 mm, the right lung takes now only a sixth part of the whole body length. A schematic reconstruction of the bronchial tree of a 28 days old *Monodelphis domestica* shows figure 32. The supply areas of the several lobar bronchi are similar as previously described in the newborn lung.

The main bronchi have a diameter of 250 µm and are lined with one-layered cuboidal epithelium (Fig. 33 a, b). A layer of smooth muscle cells is situated beneath the mucosa. For stability, cartilage surrounds the layer of muscle cells. The lobar bronchi have a diameter of 100-125 µm and their walls consist of one-layered cuboidal epithelium and a circular layer of muscle cells (Fig. 33 b). Segmental bronchi are similar in structure, however, with 60-70 µm in diameter smaller. The most distal located airways are the terminal bronchioles (Fig. 33 c). The terminal bronchioles have a diameter of 40-45 µm and are lined with one-layered cuboidal epithelium and contain only few muscle cells in their walls. The cuboidal epithelium of the distal parts of the terminal bronchioles shows a dense covering with ciliated cells (Fig.

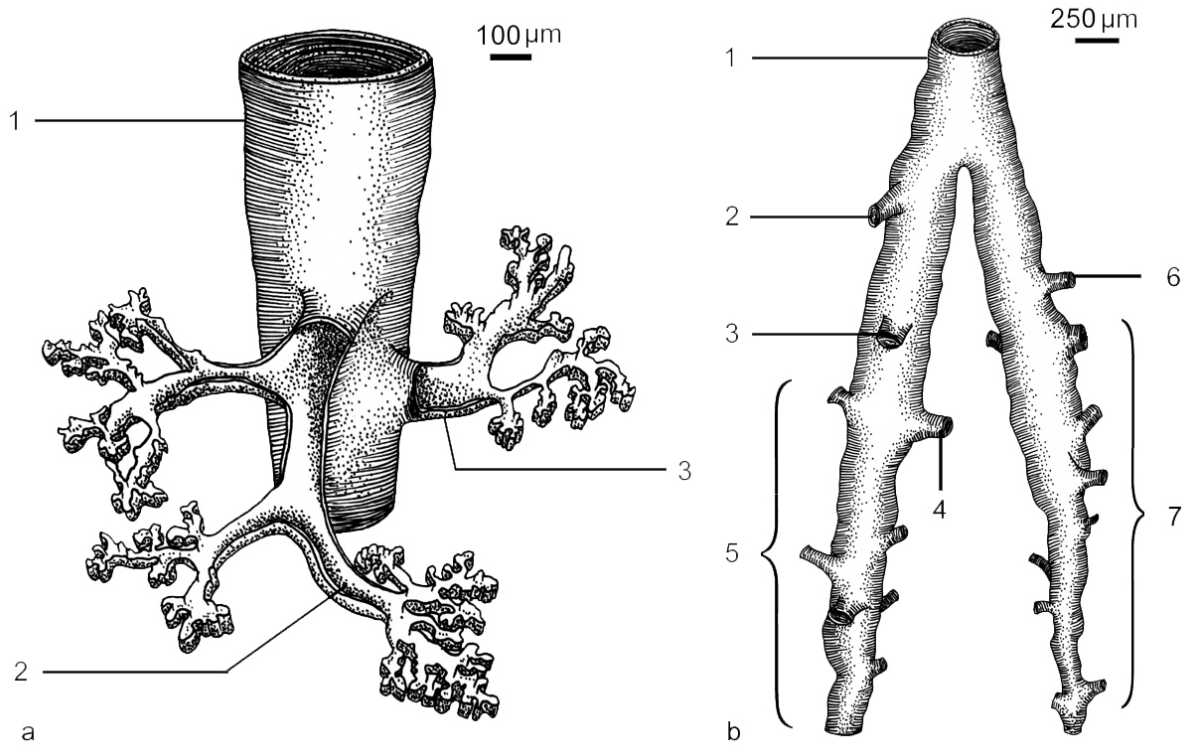

Fig. 32: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the 28 days old lung of *Monodelphis domestica*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 - inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

33 g, h). With the formation of alveoli first respiratory bronchioles develop. They are characterised by flattened epithelium with shallow depressions in their walls (Fig. 33 g).

Terminal air sacs are still present in the 28 days old *Monodelphis domestica* (Fig. 33 e). They have a diameter of 50-70 µm and are lined by the typical epithelium of flattened type I and cuboidal type II pneumocytes. At this age the formation of alveoli starts. Alveoli are formed by spouting from the walls of the air sacs (Fig. 33 c, f). In this period first distinct alveolar pouches are seen in the walls of the air sacs. The alveoli measure 40-50 µm in diameter and the lining epithelium is the same as in the air sacs (Fig. 34 a, b). The type II pneumocytes have the characteristic microvilli on their luminal surfaces and contain lamellar bodies (Fig. 34 f). They can be found singly or in pairs (Fig. 34 e). At the age of four weeks a septum with a double capillary bed is still present (Fig. 34 a). However, with the formation of alveoli additionally a septum with a single capillary bed is formed (Fig. 34 b, c). This single capillary septum ensures the gas exchange via the blood-air barrier from the both adjacent alveoli. Compared to the earlier stages, the thickness of the double capillary and single capillary septa decreases remarkably. They measure only 6.5-7.5 µm in width. The interstice of the septa is similar to the previously described stages. However, the appearance is more

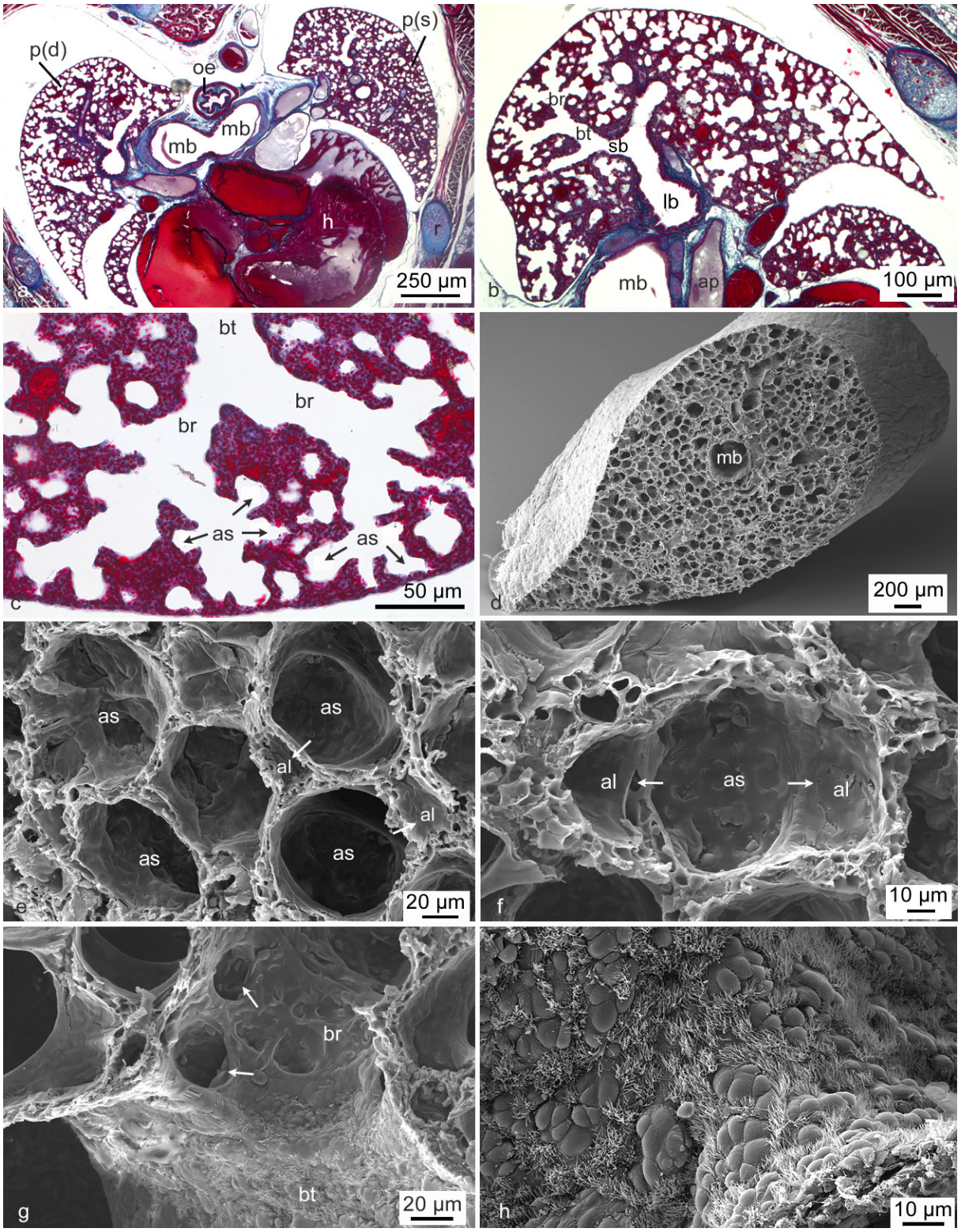

Fig. 33: Light micrographs (a-c) and scanning electron micrographs (d-h) of the 28 days old lung of *Monodelphis domestica*. The lung of the four weeks old *Monodelphis domestica* appears more subdivided and is characterised by the beginning formation of alveoli (a, b, d). The still present terminal air sacs have a diameter of 50-70 µm and the alveoli sprouting from the air sacs measure 40-50 µm in diameter (e, f; openings of alveoli are indicated by arrows). The air sacs and first alveoli branch off from terminal bronchioles, which partially start to form also alveoli on their sides and become respiratory bronchioles (b, c, g). The distal parts of the terminal bronchioles show ciliated cells (g, h). as, air sac; al, alveole; ap, pulmonary artery; bt, terminal bronchiole; br, respiratory bronchiole; h, heart; lb, lobar bronchus; mb, main bronchus; oe, oesophagus; p(d) right lung; p(s), left lung; r, rib; sb, segmental bronchus. Azan staining. Magnification is indicated by the scale bar.

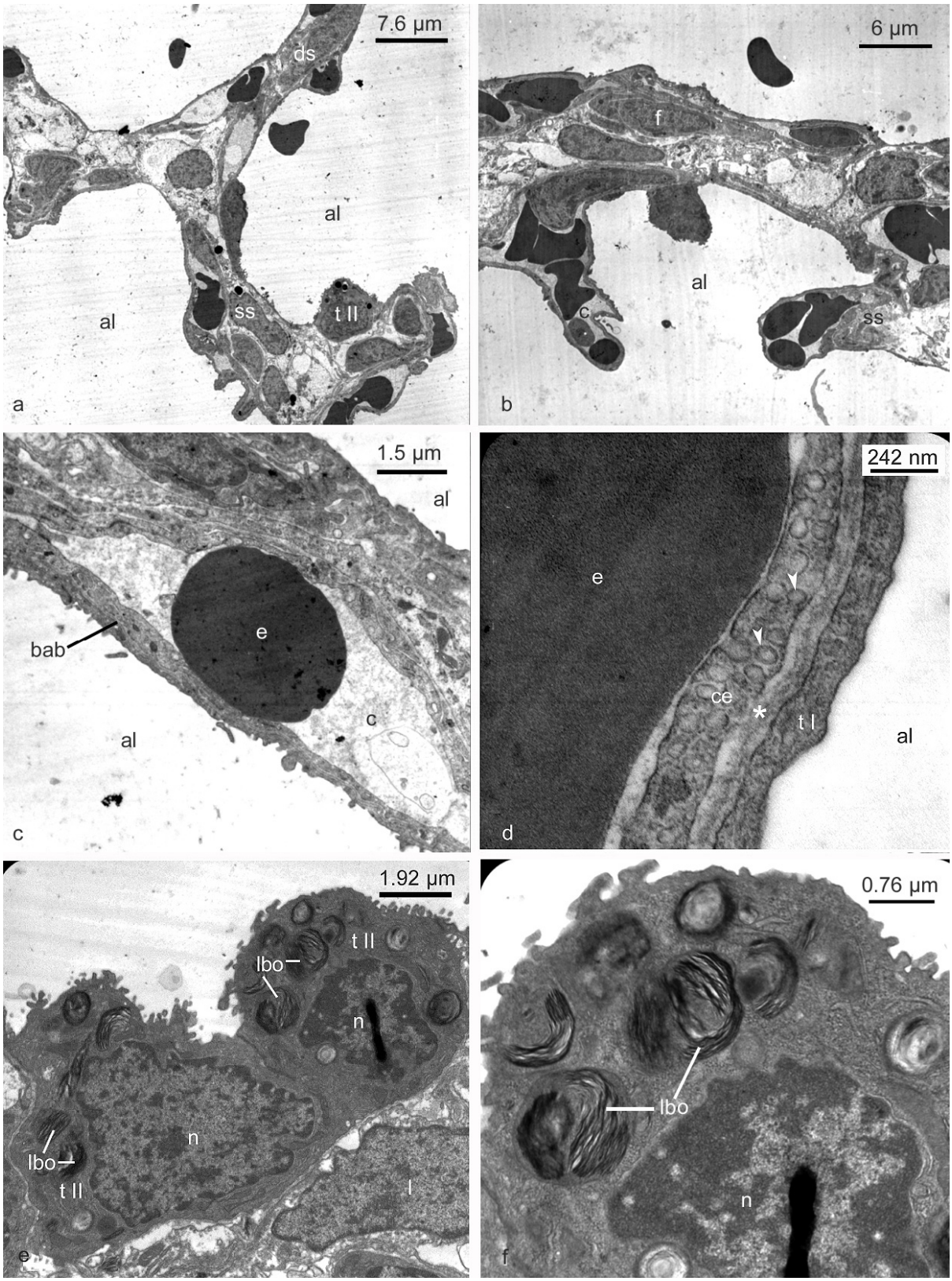

Fig. 34: Transmission electron micrographs of the 28 days old lung of *Monodelphis domestica*. At the age of four weeks the formation of alveoli begins (a, b). The alveoli are separated by a septum, composed of a single capillary network (c). The blood-air barrier shows the typical trilaminar structure of type I pneumocytes, endothelial cells and fused basal lamina of both cell types (∗) (d). The capillary endothel contains coated vesicles (arrows). The cuboidal type II pneumocytes are found singly or in pairs (e,f). al, alveole; bab, blood-air barrier; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; ds, double capillary septum; ss, single capillary septum; t I, type I cell; t II, type II cell. Magnification is indicated by the scale bar.

compact and only a few interstitial spaces are present. The blood-air barrier has the same appearance and thickness as those of the lung in younger animals (Fig. 34 d).

The lung of the 41 days old *Monodelphis domestica*

The lung of the six weeks old *Monodelphis domestica* is charcterised by a further increase in size (Fig. 21.2 c) and the proceeding formation of alveoli. The light microscopic findings are presented in figure 35. The previously described elements of the bronchial tree, main, lobar and segmental bronchi as well as terminal and respiratory bronchioles are present. The respiratory bronchioles with alveoli on their sides are found more frequently. The distal parts of the respiratory bronchioles pass into alveolar ducts which open into alveolar sacs. A special feature of this developmental stage is that sporadical alveoli are located at the walls of segmental bronchi and terminal bronchioles.

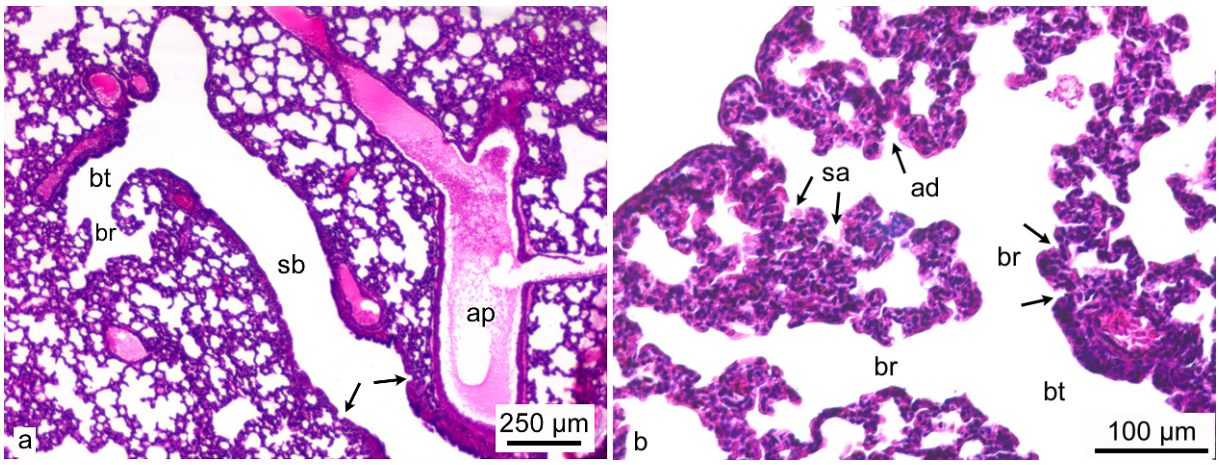

Fig. 35: Light micrographs of the 41 days old lung of *Monodelphis.* In the lung of the six weeks old *Monodelphis* the formation of alveoli is proceeding. Alveoli are more numerous. They radiate from alveolar sacs or are located at the walls of alveolar ducts and respiratory bronchioles. In addition sporadic alveoli are found at the walls of conducting airways (a, b; alveoli are indicated by arrows). ad, alveolar duct; ap, pulmonary artery; bt, terminal bronchiole; br, respiratory bronchiole; sa, alveolar sac; sb, segmental bronchus. HE staining. Magnification is indicated by the scale bar.

The lung of the 56 days old *Monodelphis domestica*

At 56 days, the air compartments of the lung parenchyma have markedly increased in number and have decreased in size. The formation of alveoli continues. The light and electron microscopic findings of the structural lung development of the 56 days old *Monodelphis* are presented in the figures 36 and 37.

The right lung measures 9.7 mm in total length. The left lung is slightly smaller with a longitudinal expansion of 8.7 mm. Associated with the general increase of body size a increase in size of the lung took place. In relation to the total body length of 69 mm, the right

lung takes now only a seventh part of the whole body length.

In consequence of the increase in total lung size also the bronchial tree is getting longer. The bronchial tree is widely ramified. It extends, starting from the main bronchus via lobar and segmental bronchi as well as terminal and respiratory bronchioles, deep into the periphery of the lung (Fig. 36 a, b).

The main bronchi have doubled their diameter to 500 µm. They are lined with one-layered cuboidal, partly columnar epithelium. The next layer consists of smooth muscle cells, separated by connective tissue. Embedded in the connective tissue seromucous glands can be found. The proximal part of the main bronchi is supported by cartilage. Cartilage is present up to the second dichotomy of the main bronchus in the right and up to the first dichotomy of the main bronchus in the left lung. The lobar and segmental bronchi have a diameter of 300-400 µm. They are lined with cuboidal epithelium and have smooth muscle cells in their walls. The terminal bronchioles have a diameter of 50-100 µm and are musculare to a lesser extent. With the progressive formation of alveoli numerous respiratory bronchioles with a diameter of 50-100 µm develop. The peripheral parts of the bronchial tree have alveoli at their sides and take part at the gas exchange. But in addition to this regulary development of the respiratory bronchioles (Fig. 36 d), numerous alveoli are present also at the walls of solely conducting airways, such as segmental bronchi and terminal bronchioles. The wall of such a segmental bronchus is comparatively thin; with only a few muscle cells.The bronchial wall is perforated from the openings of numerous alveoli (Fig. 36 c, f). This bronchus has conducting as well as respiratory function. Therefore it can be termed as “respiratory bronchus”. In the terminal bronchioles, the epithelium is cuboidal and consists of ciliated and nonciliated cells (Fig. 36 h, 37 g, h). The ciliated cells, characterised by distinctive cilia in the apical region, move secretions and trapped particles towards the pharynx and protect so the adjacent respiratory epithelium. The nonciliated cells, also called Clara cells, are unique to bronchioles and are found interspersed between the ciliated cells (Fig. 37 h).

They contain vesicles and mitochondria in their apical region and a nucleus and rough endoplasmic reticulum in the basal region. A major function attributed to nonciliated cells is the secretion of the material lining the bronchiolar lumen, such as proteins important in defense (lysozyme, antibodies).

With the proceeding formation of alveoli, the distal parts of the respiratory bronchioles pass into short alveolar ducts. They are covered with respiratory epithelium and have alveoli at their sides (Fig. 36 d). The alveolar ducts open into alveolar sacs, from which alveoli radiate into the surrounding parenchyma (Fig. 36 g). The alveoli measure 20-40 µm in diameter and are lined with squamous type I pneumocytes and cuboidal type II pneumocytes. They are

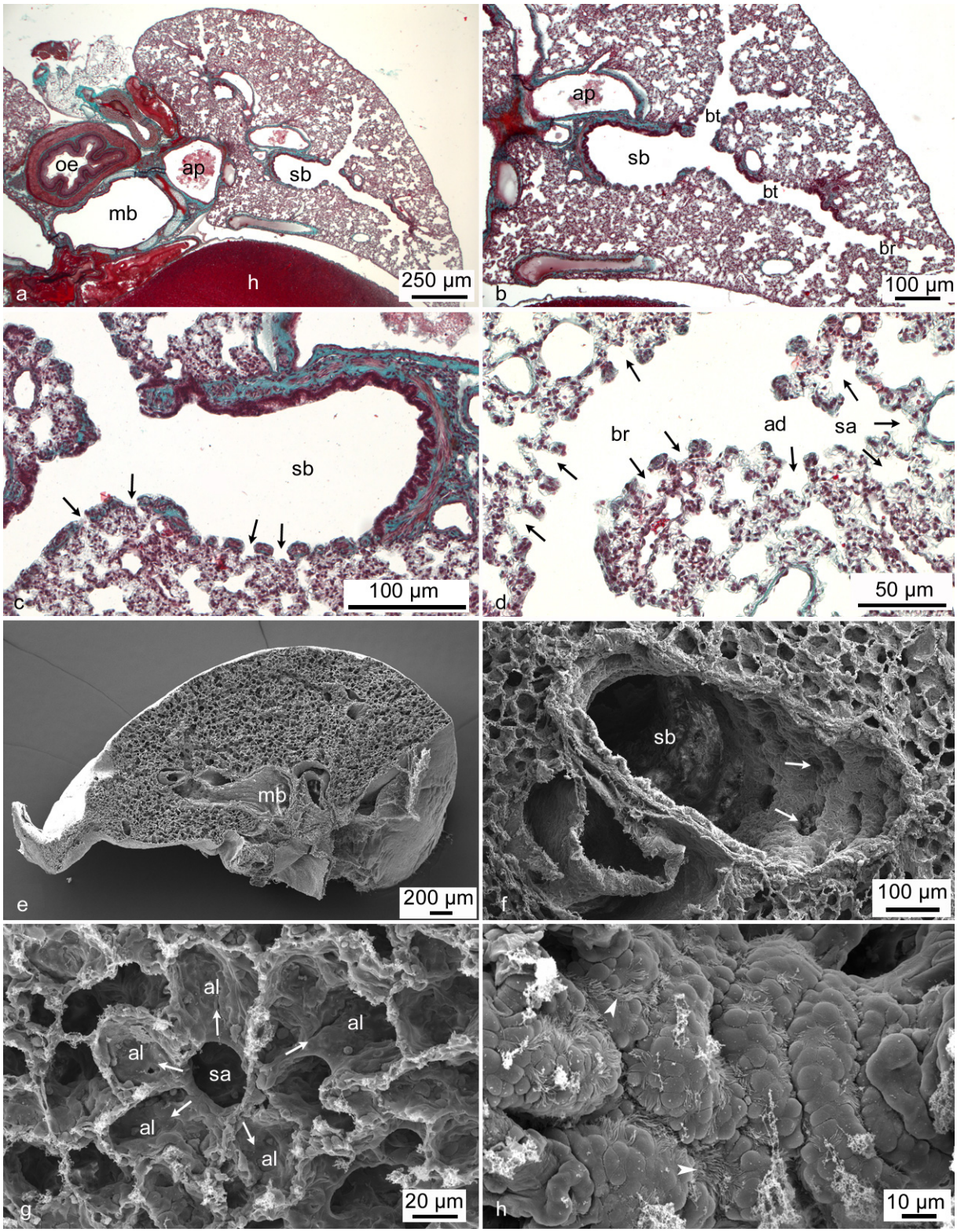

Fig. 36: Light micrographs (a-c) and scanning electron micrographs (d-h) of the 56 days old lung of *Monodelphis domestica*. The lung of the eight weeks old *Monodelphis* appears strongly subdivided and is characterised by the proceeding formation of alveoli (a, b, e). The numerous alveoli are smaller and measure 20-40 µm in diameter. With the proceeding formation of alveoli also afferent alveolar ducts and alveolar sacs develop (d, g; alveoli are indicated by arrows). Respiratory bronchioles are more frequently found. Special features are the numerous alveoli at the walls of conducting airways, such as segmental bronchi (c, f). At the distal parts of the terminal bronchioles ciliated cells can be found (h; arrows). ad; alveolar duct; al, alveole; ap, pulmonary artery; bt, terminal bronchiole; br, respiratory bronchiole; h, heart; mb, main bronchus; oe, oesophagus; sa, alveolar sac; sb, segmental bronchus. Trichrome staining. Magnification is indicated by the scale bar.

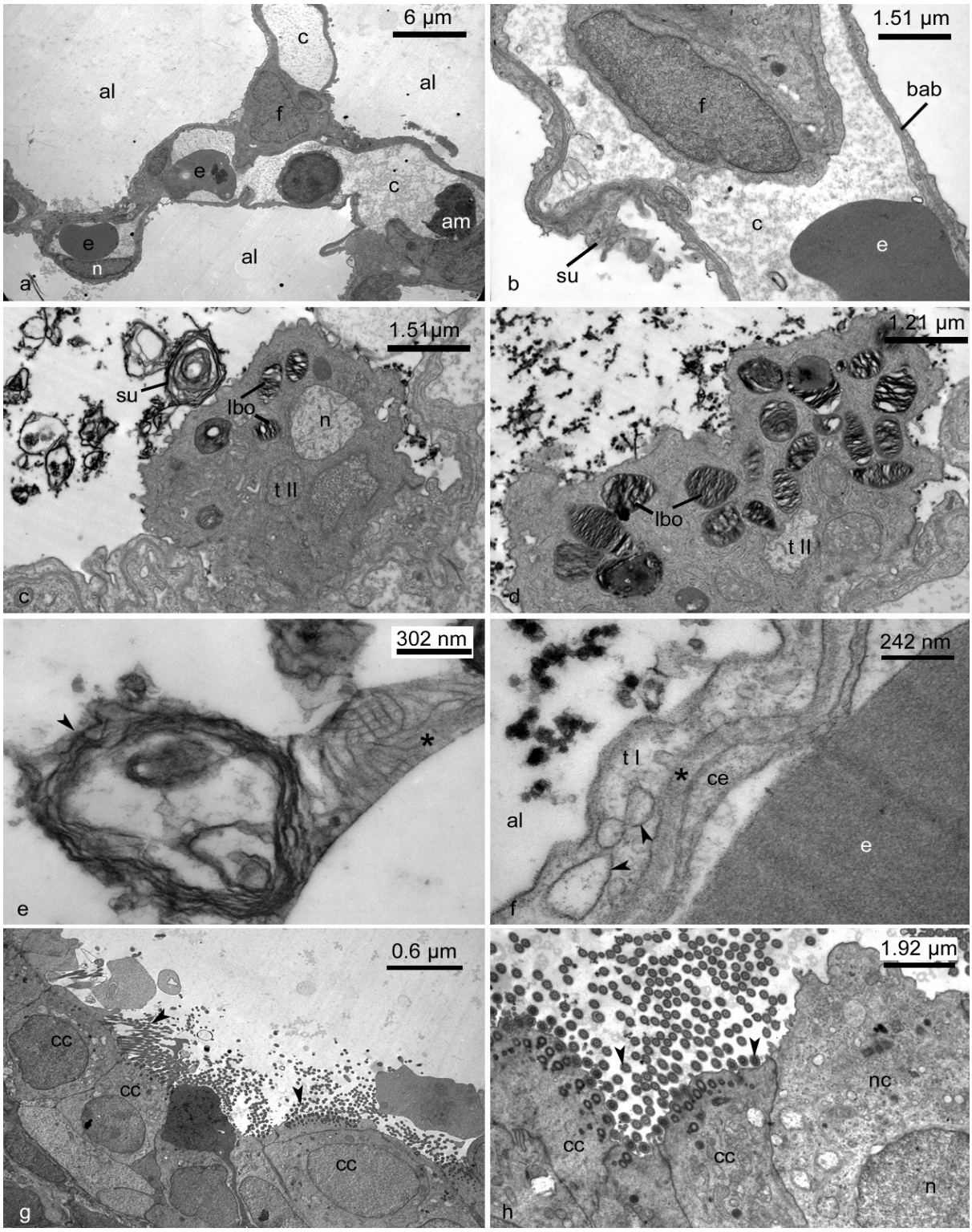

Fig. 37: Transmission electron micrographs of the 56 days old lung of *Monodelphis domestica*. At the age of eight weeks the formation of alveoli is advanced and single capillary septa are common (a, b). The septum contains a single capillary network, which has contact to both neighboring air spaces (b). Compared to earlier stages the blood-air barrier remains unchanged in structure and thickness (f; coated vesicles in type I cells indicated by arrows). The cuboidal type II pneumocytes contain numerous lamellar bodies and a huge amount of surfactant is secreted (c, d). In the air space surfactant changes its compact storage form (arrow) into a surface covering film (e, asterisk). The distal part of terminal bronchioles is covered with ciliated and nonciliated cells (g, h). al, alveole; bab, blood-air barrier; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; nc, nonciliated cell; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

separated from each other by septa with a single capillary bed (Fig. 37 a, b). The single capillary septa are thin and measure only 5-6 µm in width. The septum is dominated by the capillaries, which occupy nearly the entire septum (Fig. 37 b). Compared to earlier stages, the blood-air barrier remains unchanged in structure and thickness (Fig. 37 f). The type II pneumocytes of the eight weeks old lung have the same structure as the previously described stages; however, some type II pneumocytes contain a remarkable hugh amount of lamellar bodies (Fig. 37 d). A large quantity of surfactant in the air space of the alveoli indicates an increased secretion of surfactant from the type II pneumocytes (Fig. 37 c). The phospholipids synthesised and stored in the lamellar bodies have a compact, filamentous structure. After secretion, free in the alveolar lumen, the surfactant transforms from the lamellar structure into a loose irregular structured form that covers the alveolar surface (Fig. 37 e).

The lung of the adult (3 months) *Monodelphis domestica*

The lung parenchyma of the adult lung is strongly subdivided and the gas exchange surface area has increased (Fig. 38 c). The walls of the alveoli appear to be thinner. The histological and ultrastructural findings for the adult *Monodelphis* lung are shown in the figures 38 and 39.

The adult bronchial tree, consisting of main bronchi, lobar bronchi and segmental bronchi, is similar in structure to the previously described bronchial tree of an eight weeks old *Monodelphis*. However, the lumina of the bronchi increase in size and the walls are getting thicker. The main bronchi measure 1.5 mm in diameter. Their wall is lined with pseudostratified ciliated columnar epithelium. In the proximal part circular layers of smooth muscle cells and cartilage are present. The contraction of the bronchial musculature leads to longitudinal folds. The lobar bronchi, branching off from the main bronchus, measure 600 µm in diameter. Their walls are lined with cuboidal epithelium and consist of a thick layer of smooth muscle cells equal to those of the main bronchi. Either the lobar bronchi are conductive for a long distance in order to supply peripheral lung segments, or they divide into segmental bronchi after a short distance. The segmental bronchi measure 200-400 µm in diameter and their walls are supported by a circular layer of smooth muscle cells, which is thinner than in main or lobar bronchi. Short after branching off from the lobar bronchi, the walls of the segmental bronchi are perforated by numerous alveoli (Fig. 38 d). These irregular formations of alveoli, similar to the previously described eight weeks old stage, are widespread within the adult lung. Irregular formation of alveoli takes place also at the walls of terminal bronchioles. With the maturation of the lung, the musculature increases in the proximal parts and expands also to peripheral parts of the lung. Even terminal bronchioles

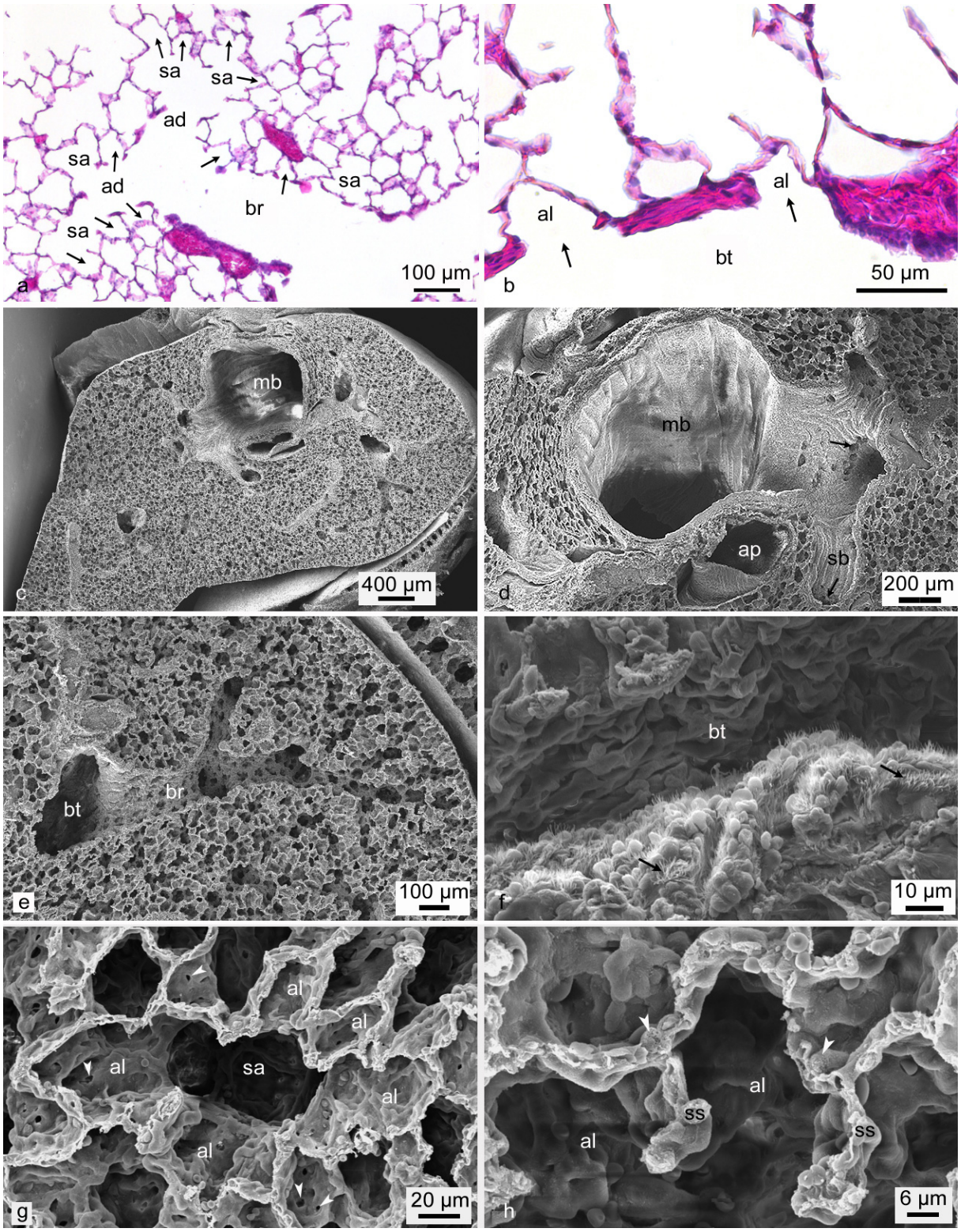

Fig. 38: Light micrographs (a, b) and scanning electron micrographs (c-h) of the adult lung of *Monodelphis domestica*. The lung parenchyma is highly subdivided and is made up of areas of thin-walled alveoli (a, c). The location of alveoli is regular at the walls of respiratory bronchiols and alveolar ducts (a, e; alveoli are indicated by arrows) but irregular at walls of segmental bronchi and terminal bronchioles (b, d; alveoli are indicated by arrows). In the periphery of the lung, alveoli radiate from alveolar sacs (g; arrowheads indicate pores of Kohn). The separation of the alveoli is made up of a single capillary septum (h; arrowheads indicate type II pneumocytes). The distal parts of the terminal bronchioles are lined with ciliated cells (f; arrows). ad; alveolar duct; al, alveole; ap, pulmonary artery; bt, terminal bronchiole; br, respiratory bronchiole; mb, main bronchus; sa, alveolar sac; sb, segmental bronchus; ss, single capillary septum. HE staining. Magnification is indicated by the scale bar.

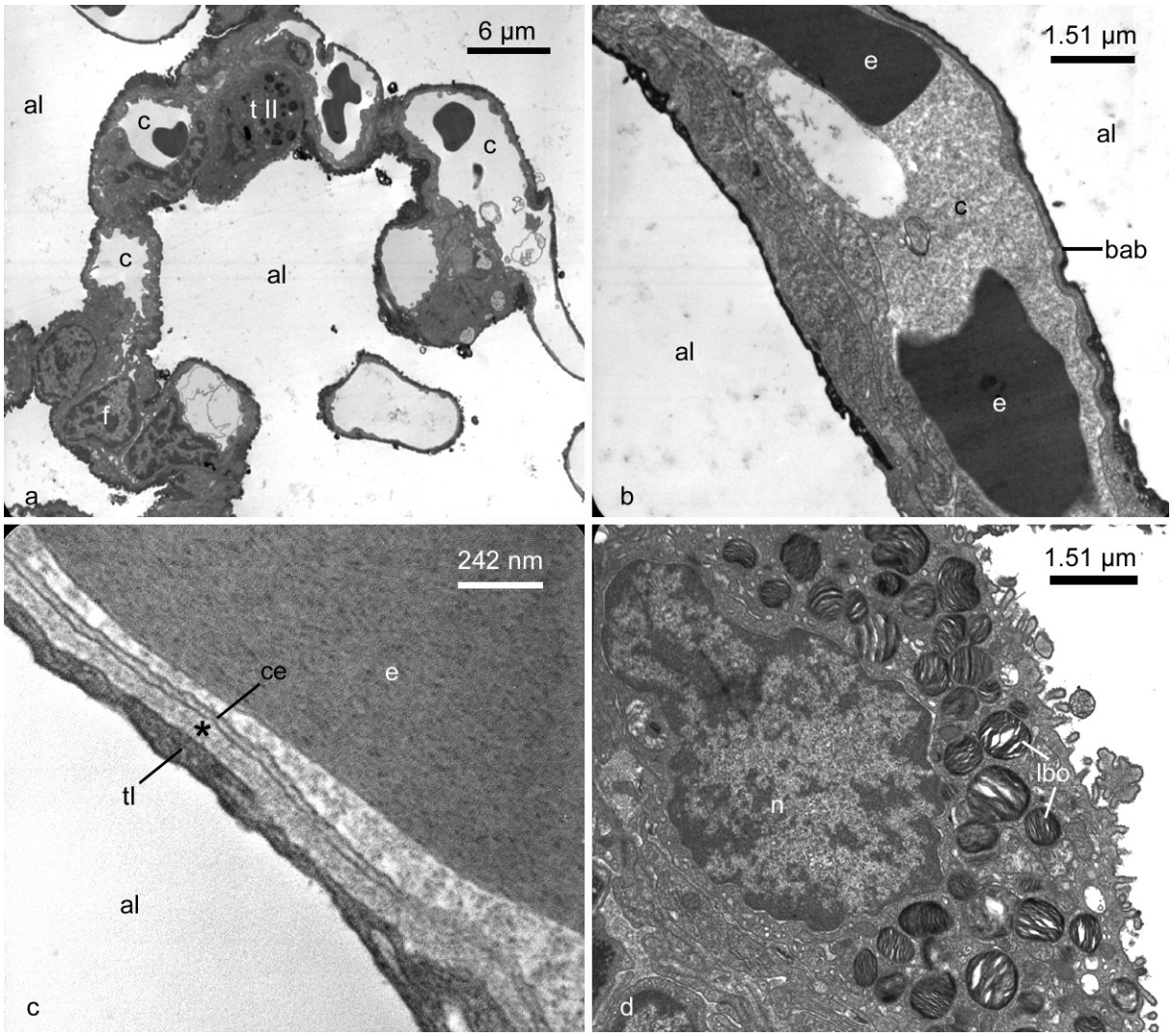

Fig. 39: Transmission electron micrographs of the lung of the adult *Monodelphis domestica*. The alveoli are separated by a septum, spanned by a single capillary network (a, b). The blood-air barrier consists of an endothelial layer, a basal lamina (asterisk), and a epithelial layer (c). The diffusion barrier is thin and has now a thickness of 150 nm. The type II pneumocytes contain numerous lamellar bodies, what indicate an increased surfactant production (d). al, alveole; bab, blood-air barrier; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

have a thin layer of smooth muscle cells inside their walls (Fig. 38 b). The terminal bronchioles are lined with cuboidal epithelium and are characterised by the typical ciliated cells (Fig. 38 f). Terminal and respiratory bronchioles are the most distally located airways and measure 200 µm in diameter (Fig. 38 e). The respiratory bronchioles have regular alveoli at their sides. From the respiratory bronchioles smaller alveolar ducts (100 µm) branch off. They open into alveolar sacs, from which alveoli radiate in a concentric way (Fig. 38 a, g).

The alveoli measure 50-70 µm in diameter. In comparison with earlier stages they have slightly increased in size. In the interalveolar walls, interalveolar pores or pores of Kohn are numerous and present throughout the adult lung. The pores of Kohn (Fig. 38 g, arrowheads) form a connection between adjacent alveoli. The single capillary septa between the alveoli

are very thin, they measure only 6 µm in width (Fig. 38 h, 39 a). The interstice, composed of connective tissue and fibroblasts, is reduced to a great extent. A centrally located capillary occupies the septum almost entirely (Fig. 39 b). In some areas, on one side of the septum, a thin layer of interstice intervenes between the endothelial cell and the epithelial cell and their corresponding basal lamina.

The blood-air barrier of the adult lung is in composition and structure similar to previously described stages and consists of capillary endothelium, alveolar epithelium and a fused basal lamina of both cell types (Fig. 39 c). However, the blood-air barrier becomes thinner with maturity. The diffusion barrier of the adult lung has a thickness of 150-170 µm. The number of type II pneumocytes has decreased compared to earlier stages. They are to find usually singly, located at the alveolar surface at septal junctions (Fig. 38 h). Inside the type II pneumocytes, the high amount of lamellar bodies is remarkable (Fig. 39 d). That points out an increased production of surfactant during the continuing formation of alveoli.

3.2.1.2 Macropus eugenii

The lung development of *Macropus eugenii* was investigated before by Runciman (1994) and Cehun (1994). These studies focussed on the peripartum and cardiorespiratory development of the tammar wallaby. For the comparative approach of my dissertation project, the postnatal lung development of *Macropus eugenii* was histologically examined. Concerning the ultrastructure of the lung, the previous studies are a valuable supplement.

The neonate of *Macropus eugenii* is born at an embryonic stage and remains for a long postnatal period of 105 days in the maternal pouch. During this time the lung maturation takes place. Surveys of the structural changes during the postnatal lung development of Macropus eugenii give the figures 40.1 and 40.2.

At birth the morphology of the *Macropus eugenii* lung is adapted to air breathing. The neonatal lung is at the terminal sac stage of development. A primitive bronchial tree, consisting of main and lobar bronchi, leads directly to the relatively few large terminal air sacs, where the gas exchange takes place (Fig. 40.1 a). The development of the lung during the early postnatal phase can be summarised as a process of proliferation. The epithelial and mesenchymal tissues proliferate and start forming septal crests that subdivide the terminal air sacs into smaller saccules (Fig. 40.1 b, c). With incorporation of air sacs next to established bronchi, new bronchi are formed. These segmental bronchi enlarge the developing bronchial tree. At the age of 24 days an over-all size increase of the lung takes place. The terminal air sacs become smaller, due to a continuous subdivision of the air sacs by septal outgrowths (Fig. 40.1 d). In the ensuing period, the most noticeable development of

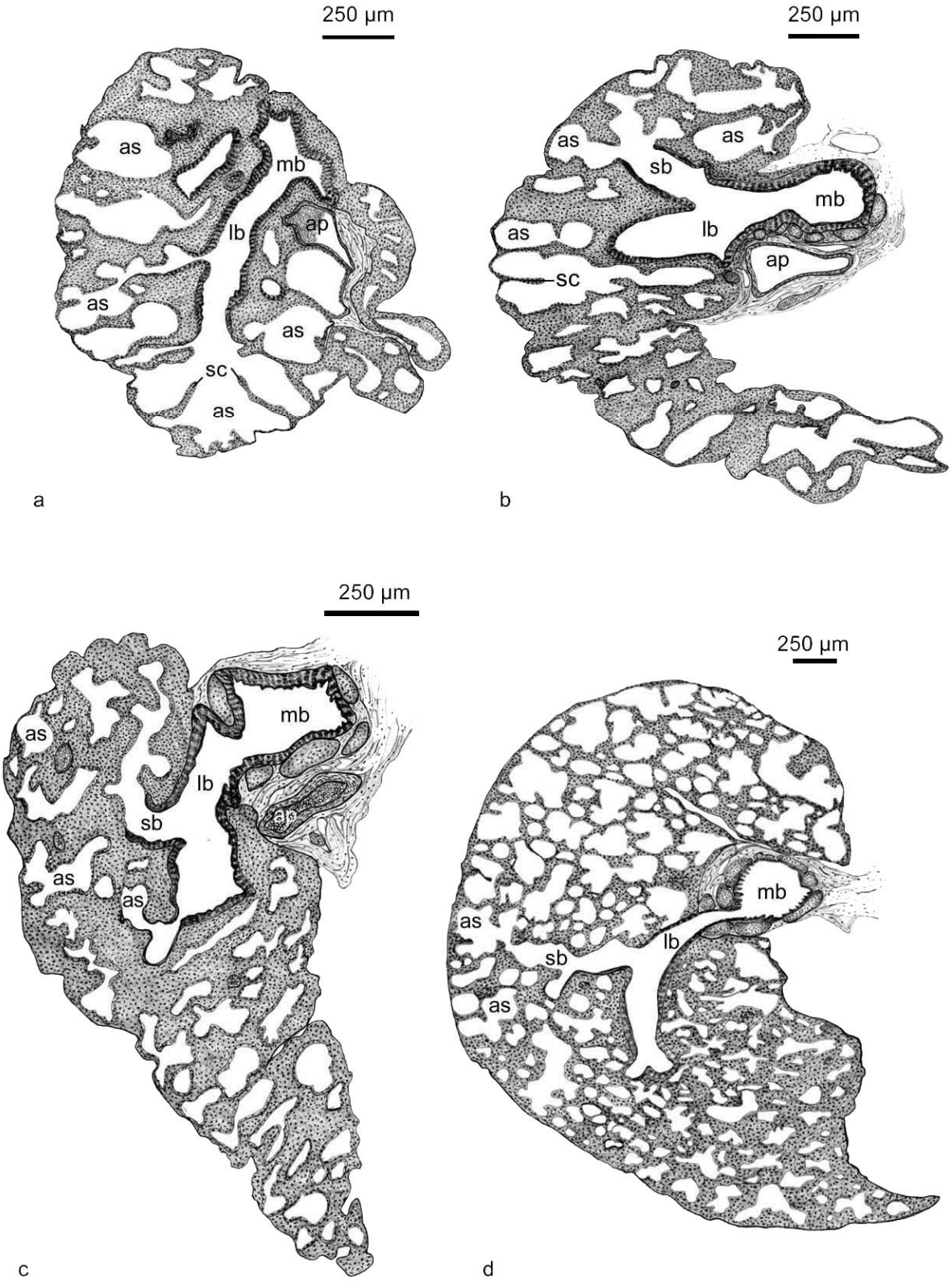

Fig. 40.1: Lung structure of *Macropus eugenii* during the postnatal development. Original drawings from histological sections of the right lung of a neonate (a) and a 5 (b), 10 (c) and 24 days (d) old *Macropus eugenii.* ap, pulmonary artery; as, air sac; lb, lobar bronchus; mb, main bronchus; sb, segmental bronchus; sc, septal crest. Magnification a-c 100 x, d 50 x.

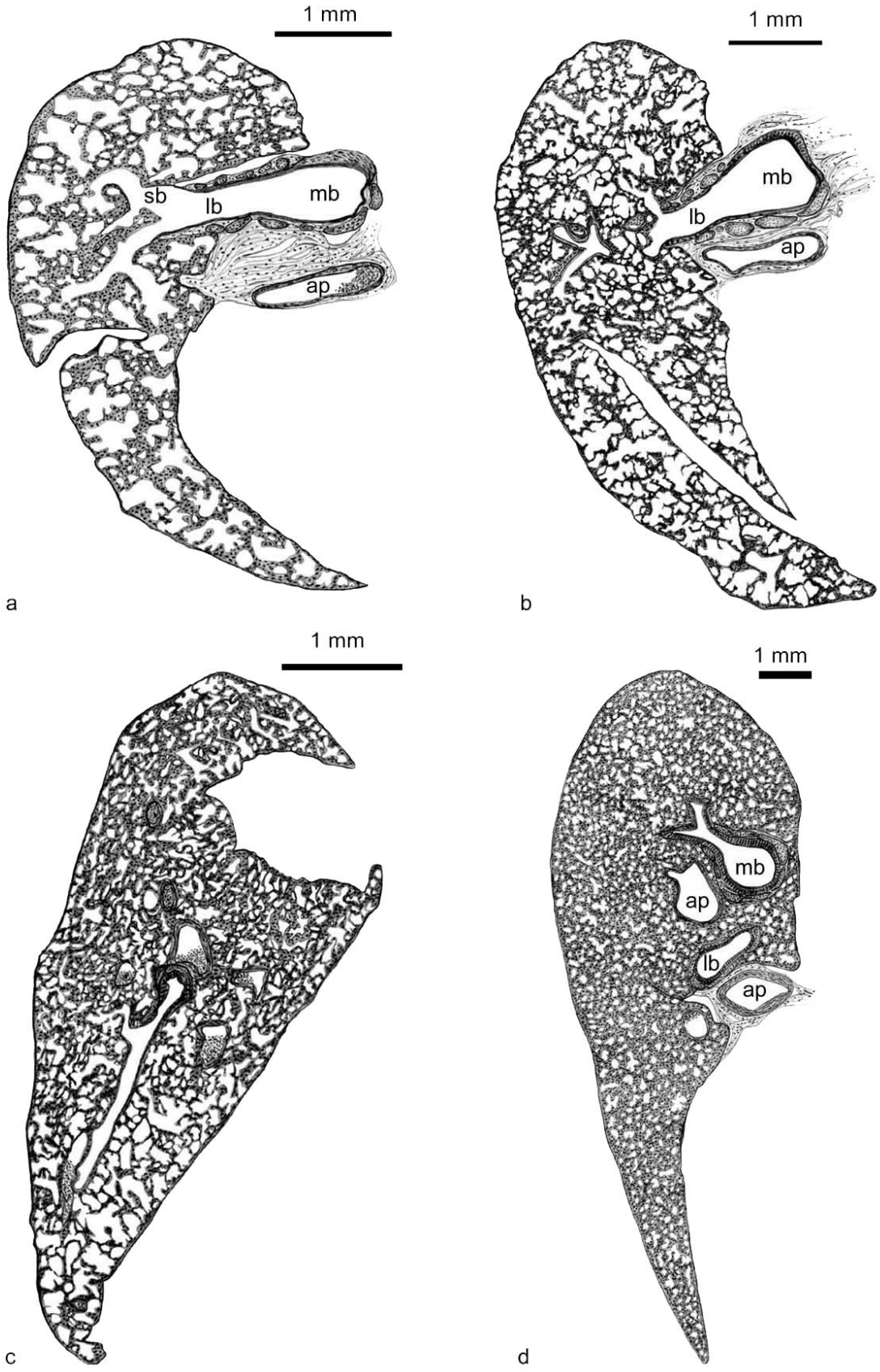

Fig. 40.2: Lung structure of *Macropus eugenii* during the postnatal development. Original drawings from histological sections of the right lung of a 42 (a), 65 (b), 98 (c) and 142 days (d) old *Macropus eugenii.* ap, pulmonary artery; as, air sac; lb, lobar bronchus; mb, main bronchus; sb, segmental bronchus. Magnification a-c 25 x, d 12.5 x.

the lung of *Macropus eugenii* is an increase in the number and overall reduction in size of the terminal air sacs (Fig. 40.2 a). Numerous septal crests that vary in height and thickness cause a more irregular shape of the air sacs. The first formation of alveoli can be observed at an age of 65 days (Fig. 40.2 b). Beside double capillary septa, characteristic for the air sac stage, first single capillary septa, characteristic for the alveolar stage, are present. The following period is characterised by a slowly increase of the number of alveoli (40.2 c). At 142 days, the air compartments of the lung parenchyma of *Macropus eugenii* have markedly increased in number and have decreased in size (Fig. 40.2 d). The typical adult lung structure with respiratory bronchioles and alveolar sacs is present. The bronchial tree is widely ramified and the larger bronchi are supported by cartilage.

The succeeding detailed description of the lung development of *Macropus eugenii* is subdivided in the postnatal developmental stages examined.

The lung of the neonatal *Macropus eugenii*

The lung of the newborn *Macropus eugenii* is characterised by prominent smooth walled airways that extend to few large terminal air sacs. The histological findings for the neonatal lung of *Macropus eugenii* are shown in figure 41. In the neonate, the right and the left lung have nearly the same size with a longitudinal expansion of 3.3 mm. Relating to the CRL of 13 mm the right lung takes a fourth of the whole body length. The external aspects are nearly the same in both lungs, however, the arrangement of the pulmonary lobes differ. The left lung is partially divided into a small middle and a larger inferior lobe. The right lung is incompletely partitioned into superior, middle and inferior lobe, and an acessory lobe at the lateral side caudal of the heart.

From the main bronchus several lobar bronchi branch off, which either extend to the pleural surface (Fig. 41 b) or branch into smooth-walled channels, which open directly into large air sacs (Fig. 41 a). The neonatal bronchial tree with the branching of lobar bronchi is equivalent to that described in the five days old lung of *Macropus eugenii*. The main bronchi measure 200 µm in diameter. The wall of the most proximal part of the main bronchus is lined with a pseudostratified ciliated columnar epithelium. Beneath a layer of one to three smooth muscle cells is lying. In the proximal part cartilage follows beneath the muscular layer and stabilises the lumen of the main bronchus. Cartilage is present only in the extrapulmonar part of the main bronchus, until the bronchus enters the lung in the hilar region. The more distal part of the main bronchus as well as the lobar bronchi, which have a diameter of 120-150 µm, are lined with simple columnar or cuboidal epithelium and are supported by a circular layer of smooth muscle cells.

The large terminal air sacs measure 150-300 µm in diameter. They are smooth-walled with a

few septal crests protruding from the septa (Fig. 41 b, c). The air sacs are lined mainly with squamous type I pneumocytes, but also type II pneumocytes can be found interspersed between the type I pneumocytes. The ultrastructure of the tammar wallaby lung was not investigated in this study, however the results of Runciman (1994) and Cehun (1994) confirm a similar structure as found in the previously described neonatal *Monodelphis domestica.* The air sacs are separated from each other by a thick septum, which contains a double capillary network. Each air sac has its own capillary bed that is separated from the capillary bed of the adjacent air sac (Fig. 41 d). The septa forming the walls of the air sacs vary in thickness. The centrally located septa are thicker (30-40 µm) than those in the periphery (20 µm) of the lung (Fig. 41 b). The erythrocytes in the capillaries are still nucleate at birth.

The lung of the five days old *Macropus eugenii*

The lung structure of the five days old *Macropus eugenii* is similar to that of the newborn tammar wallaby. The subdivision of the large terminal air sacs by septal crests protruding from the septa progresses (Fig. 41 g). The histological findings of the lung structure of the five days old *Macropus eugenii* are presented in figure 41.

The longitudinal expansion of the right lung is 5.4 mm, whereas the left lung measures 4.9 mm in length. Compared to the neonatal lung a size increase of both lungs took place. The bronchial tree (main and lobar bronchi) of the right lung shows up to 30 dichotomies, the bronchial tree of the left lung divides up to 25 times. A schematic reconstruction of the bronchial tree of the lung of a five days old *Macropus eugenii* is presented in figure 42. In the right lung the first branch of the lateral side of the main bronchus supplies the superior lobe. The next branch at the ventral side of the main bronchus supplies the middle lobe. On the same height, a bronchus for the supply of the accessory lobe is branching off medial from the main bronchus. In the inferior part of the main bronchus several bronchi of different size follow for the supply of the inferior lobe. In the left lung, the first bronchus, branching off from the lateral side of the main bronchus, is responsible for the supply of the superior and middle part of the left lung. All other following branches are for the supply of the larger inferior lobe of the left lung. Compared to the newborn lung, the conducting airways did not appear to change structurally in the first five days of life. After branching off from the main bronchus, the lobar bronchi give rise to short afferent smooth-walled channels or lead directly to the large terminal air sacs in the periphery of the lung (Fig. 41 f).

Due to a continuous subdivision of the saccules by septal outgrowths, the terminal air sacs become slightly smaller (Fig. 41 e, g). They measure now 100-250 µm in diameter. The walls of the air sacs are composed of double capillary septa, which have a thickness of 10-20 µm. Superficial capillaries are found on both sides of the septa (Fig. 41 h).

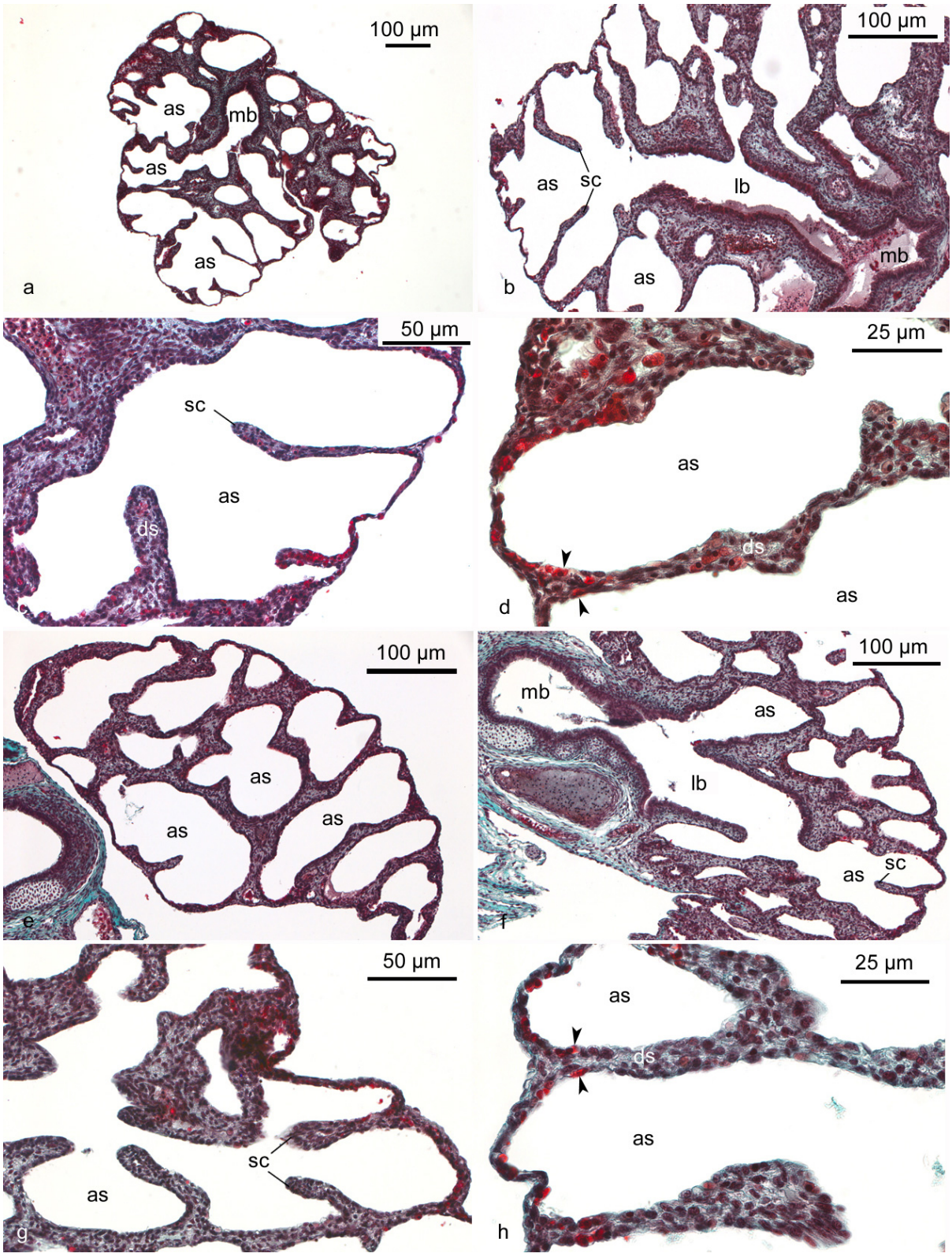

Fig. 41: Light micrographs of the neonatal (a-d) and the five days old (e-h) lung of *Macropus eugenii*. In the neonatal and five days old lung, the smooth walled airways can be seen extending to the large terminal air sacs at the pleural surface (a, b, f). Numerous septal crests arise from the walls of the terminal air sacs and subdivide the large terminal air sacs (c, g). The air sacs are separated from each other by thick septa. These septa consist of a double capillary bed (d, h; arrowheads). as, air sac; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sc, septal crest. Trichrome staining. Magnification is indicated by the scale bar.

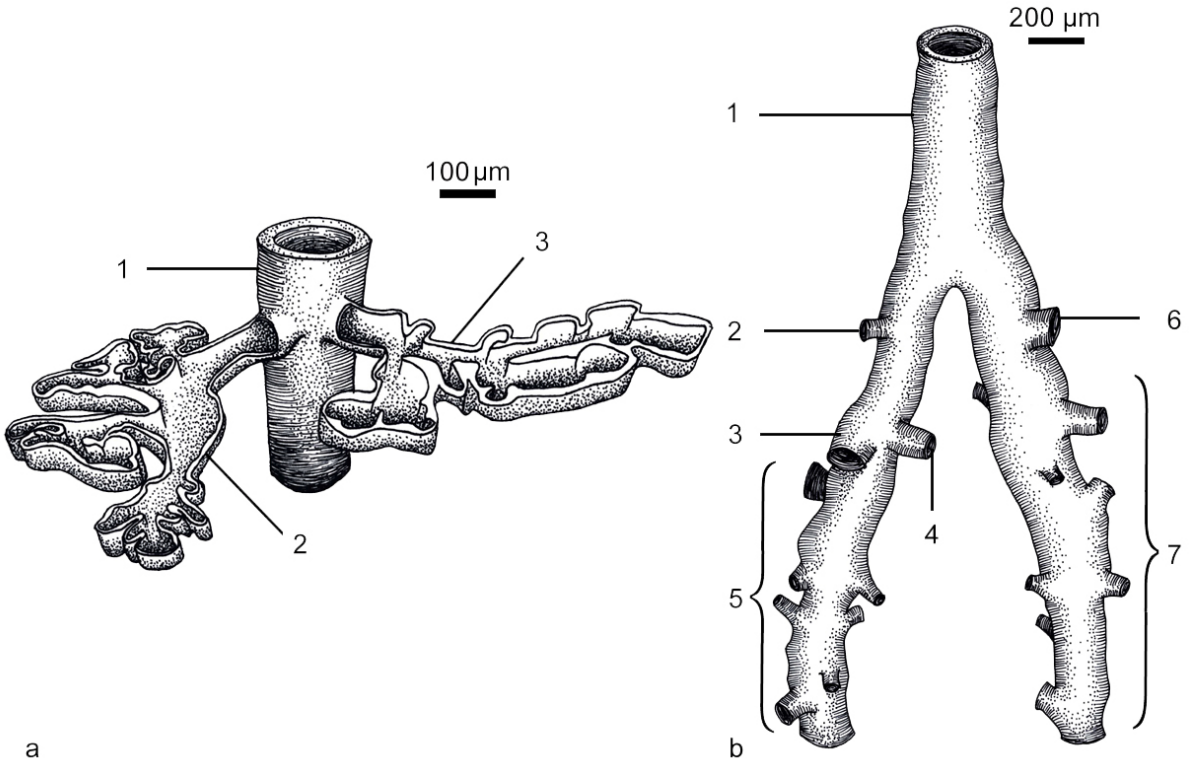

Fig. 42: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the 5 days old lung of *Macropus eugenii*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 – inferior lobe bronchi. Magnification 50 x.

The lung of the ten days old *Macropus eugenii*

At the age of ten days, the lung of *Macropus eugenii* appears more mature, well vascularised and distinctly organized. The mesenchyme has proliferated and the stroma between the air sacs shows a solid structure (Fig. 43 a). The histological findings of the lung structure of the ten days old *Macropus eugenii* are presented in the figure 43.

The bronchial tree develops. Beside the main and lobar bronchi, segmental bronchi enlarge the system of conducting airways (Fig. 43 b). The main bronchi measure 400 µm in diameter in the proximal parts and approximately 200 µm in the more distal parts. The wall of the main bronchus consists of cartilage up to the second dichotomy (middle lobe bronchus of the right lung). The structure of the larger airways is similar to that described in the newborn lung. The lining of the airways consists of a pseudostratified ciliated columnar epithelium in the main bronchi and of simple cuboidal epithelium in the lobar and segmental bronchi. In addition, the main and lobar bronchi are supported by a thin layer of smooth muscle cells. Due to a continuous outgrowth of septal crests, the large terminal air sacs become more and more subdivided (Fig. 43 c). Beside large terminal air sacs of 100-200 µm in diameter, also small air sacs of 50 µm can be found (Fig. 43 d). All the septa present, regardless of whether they

are primordia or newly formed, have a double capillary network (Fig. 43 d). The erythrocytes within the capillaries are no longer nucleate at the age of ten days.

The lung of the 24 days old *Macropus eugenii*

The most obvious structural change in the 24 days old lung of *Macropus eugenii* consists in the increase in the number and the overall reduction in size of the terminal air sacs. The histological findings of the lung structure of the 24 days old *Macropus eugenii* are presented in the figure 43 and a schematic presentation of the bronchial tree is shown in figure 44. The longitudinal expansion of the right lung is 9.7 mm, whereas the left lung measures 8.9 mm in length. Compared to the lung of the five days old *Macropus eugenii* a doubling of the lung size took place.

The bronchial tree is ramified and leads with main, lobar and segmental bronchi deep into the periphery of the lung (Fig. 43 f). The segmental bronchi branch into smaller bronchioles. From the terminal bronchioles smooth-walled channels branch off and open into terminal air sacs (Fig. 44 a). The branching pattern and the areas of supply of the lobar bronchi are conform to that described in the five days old lung (Fig. 44 b). However, in association with the overall size increase of the lung, the bronchial tree increases in total length and the distances between the outlets of the lobar bronchi elongate.

The main bronchi measure in the proximal parts 500 µm in diameter and 250 µm in diameter in the more distal parts. The cartilage in the hilar region of the main bronchi extends further distally, so that cartilage is also present up to the outlets of the inferior lobe bronchi. Likewise the most proximal parts of the larger lobar bronchi are supported by cartilage. The remaining composition of the larger airways is similar in main, lobar and segmental bronchi. A thin layer of smooth muscle cells is lying beneath the columnar or cuboidal epithelium which lines the airways. A pseudostratified ciliated columnar epithelium lines the main airways beyond the point where the bronchi are supported by cartilage. Where terminal bronchioles branch into smooth-walled channels or open directly into air sacs, a transition from conducting to respiratory epithelium takes place.

The subdivision of the large terminal air sacs proceeds and a variety of air sac sizes can be observed (Fig. 43 e). The larger air sacs measure 100-150 µm in diameter and are characterised by numerous septal outgrowths, which subdivide the air sacs and give them an irregular shape (Fig. 43 g). However, resulting from the subdivision, also smaller smooth-walled air sacs of 50 µm in diameter are present. The septa separating the air sacs have a double capillary bed (Fig. 43 h). The septa become gradually thinner and have a thickness of 10-15 µm in the 24 days old lung.

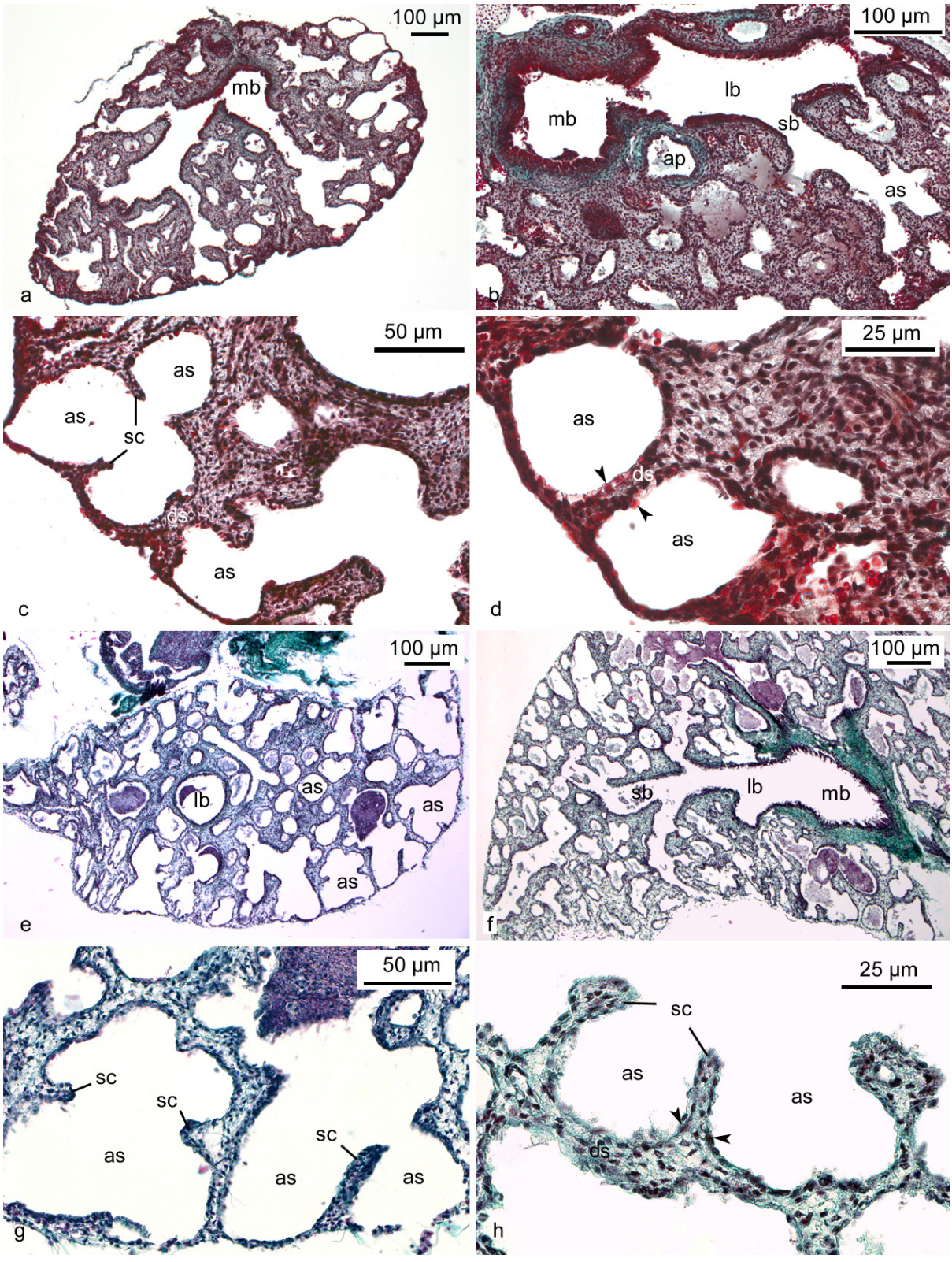

Fig. 43: Light micrographs of the 10 days (a-d) and the 24 days old (e-h) lung of *Macropus eugenii*. In the 10 and 24 days old lung, the bronchial tree develops. In addition to main and lobar bronchi, segmental bronchi extend far into the lung periphery (a, b, f). An increasing proliferation of the mesenchymal tissue gives the lung a solid appearance. The large terminal air sacs are subdivided by numerous septal crests, which arise from the walls of the terminal air sacs (c, g). Thus in addition to the large air sacs, also smaller air sacs can be found (d, h). The septa separating the air sacs from each other are double capillary septa (d, h; arrowheads indicate capillaries). as, air sac; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sb, segmental bronchus; sc, septal crest. Trichrome staining. Magnification is indicated by the scale bar.

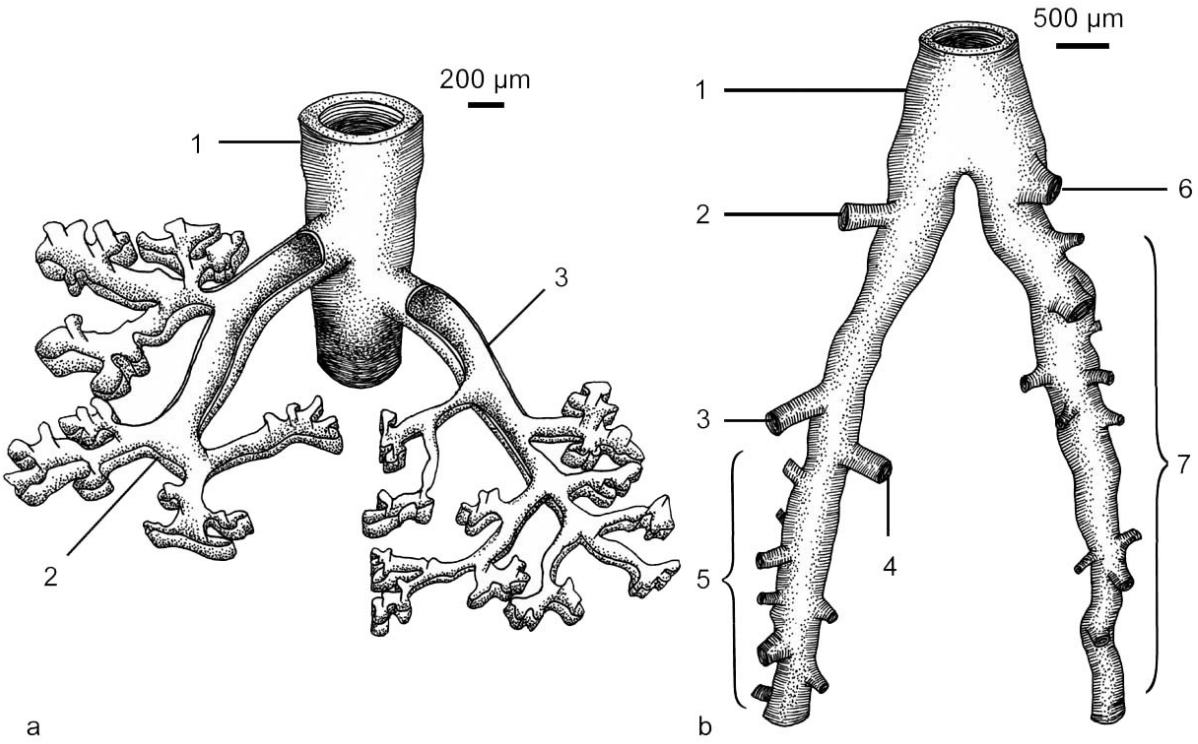

Fig. 44: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the 24 days old lung of *Macropus eugenii*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 – inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

The lung of the 42 and 55 days old *Macropus eugenii*

The lungs of the 42 and 55 days old *Macropus eugenii* are characterised by a further subdivision of the air sacs and a thinning of the septa (Fig. 45 a). Figure 45 shows the histological findings of the lung structure of the 42 and 55 days old *Macropus eugenii.* The right lung of the 42 days old *Macropus eugenii* measures 12.3 mm in length, whereas the left lung has a longitudinal expansion of 11.5 mm. In relation to the CRL of 49 mm the right lung takes still a fourth of the whole body length.

The bronchial tree of the 42 and 55 days old lung is ramified and shows many dichotomies. The course and the structure of the main airways are similar to the previously described bronchial tree of the 24 days old lung (Fig. 44 b). In the 42 and 55 days old tammar wallaby lung, a development of the terminal portions of the airways can be observed. The segmental bronchi measure 100-150 µm in diameter. They are lined with a simple cuboidal epithelium and a thin layer of smooth muscle cells supports the bronchial walls. From the segmental bronchi, smaller terminal bronchioles with a diameter of 50-70 µm branch off. These terminal bronchioles are lined with simple cuboidal epithelium and lead into the periphery of the lung (Fig. 45 c, e). The terminal bronchioles branch into numerous smooth-walled channels, which

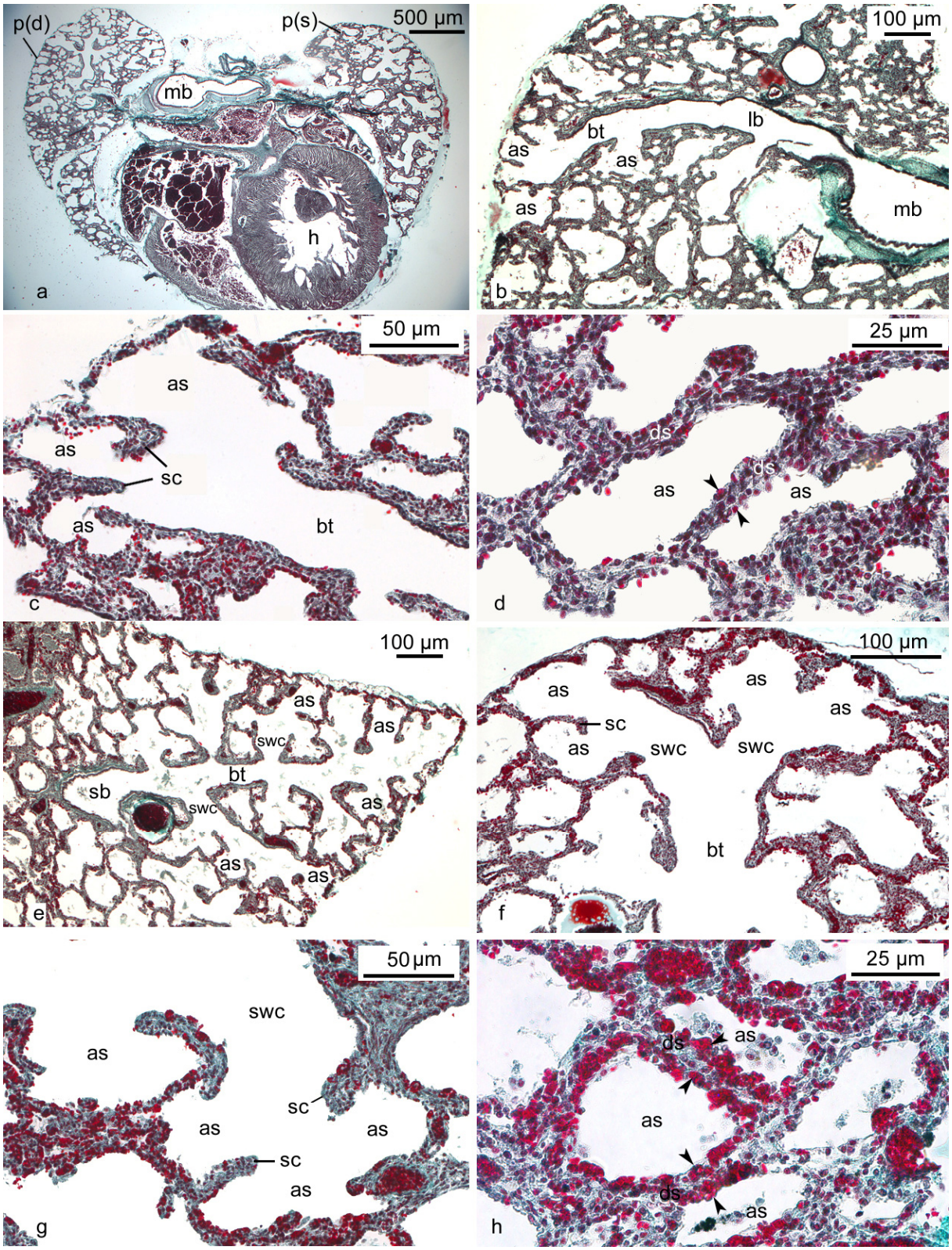

Fig. 45: Light micrographs of the 42 days (a-d) and the 55 days old (e-h) lung of *Macropus eugenii*. The 42 and 55 days old lung is characterised by the progressive subdivision of terminal air sacs and the thinning of the septa. The bronchial tree is advanced and the conducting airways lead deep into the periphery of the lung (b, e). The terminal air sacs vary in size. A few large air sacs with an irregular shape, but mainly numerous smooth-walled small air sacs are present (c, f, g). The septa between the air sacs are structurally still septa with a double capillary bed (d, h; arrows). as, air sac; bt, terminal bronchiole; h, heart; lb, lobar bronchus; mb, main bronchus; p(d), right lung (pulmo dextra); p(s), left lung (pulmo sinistra); ds, double capillary septum; sb, segmental bronchus; sc, septal crest; swc, smooth-walled channel. Trichrome staining. Magnification is indicated by the scale bar.

are lined with respiratory epithelium. These short channels open into the terminal air sacs (Fig. 45 e, f, g).

The subdivision of the terminal air sacs continues and the number of air sacs increases. There are still large air sacs of 100-150 µm, but also many small air sacs of 50 µm. The double capillary septa decrease further in thickness and measure now 5-10 µm in width (Fig. 45 d, h).

The lung of the 65 and 71 days old *Macropus eugenii*

The main feature of the lungs of the 65 and 71 days old *Macropus eugenii* is the beginning formation of alveoli. A schematic presentation of the bronchial tree of the 65 days old lung illustrates figure 46. The histological findings of the lung structure of the 65 and 71 days old *Macropus eugenii* are presented in figure 47.

At the age of 71 days, the right lung measures 19.5 mm in total length, whereas the left lung is slightly smaller in size, with a total length of 17.5 mm. With that a further size increase of the lung took place.

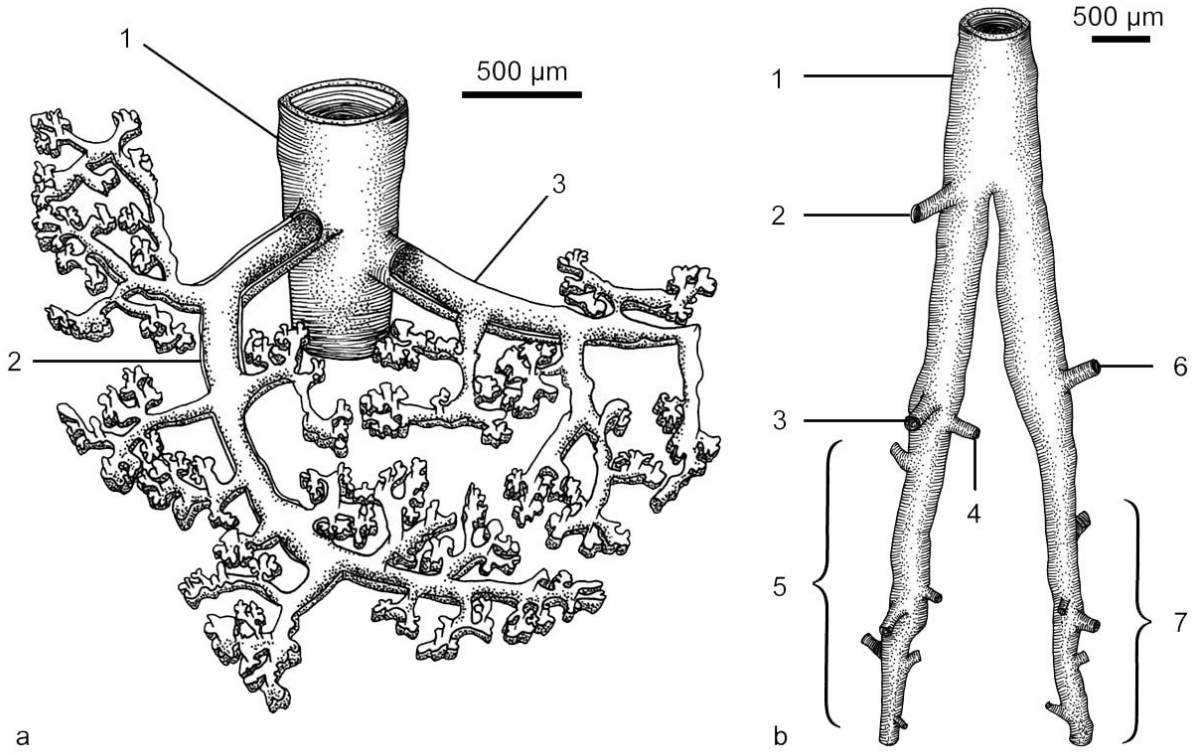

Fig. 46: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the 65 days old lung of *Macropus eugenii*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 – inferior lobe bronchi. Magnification a: 25 x; b: 12.5 x.

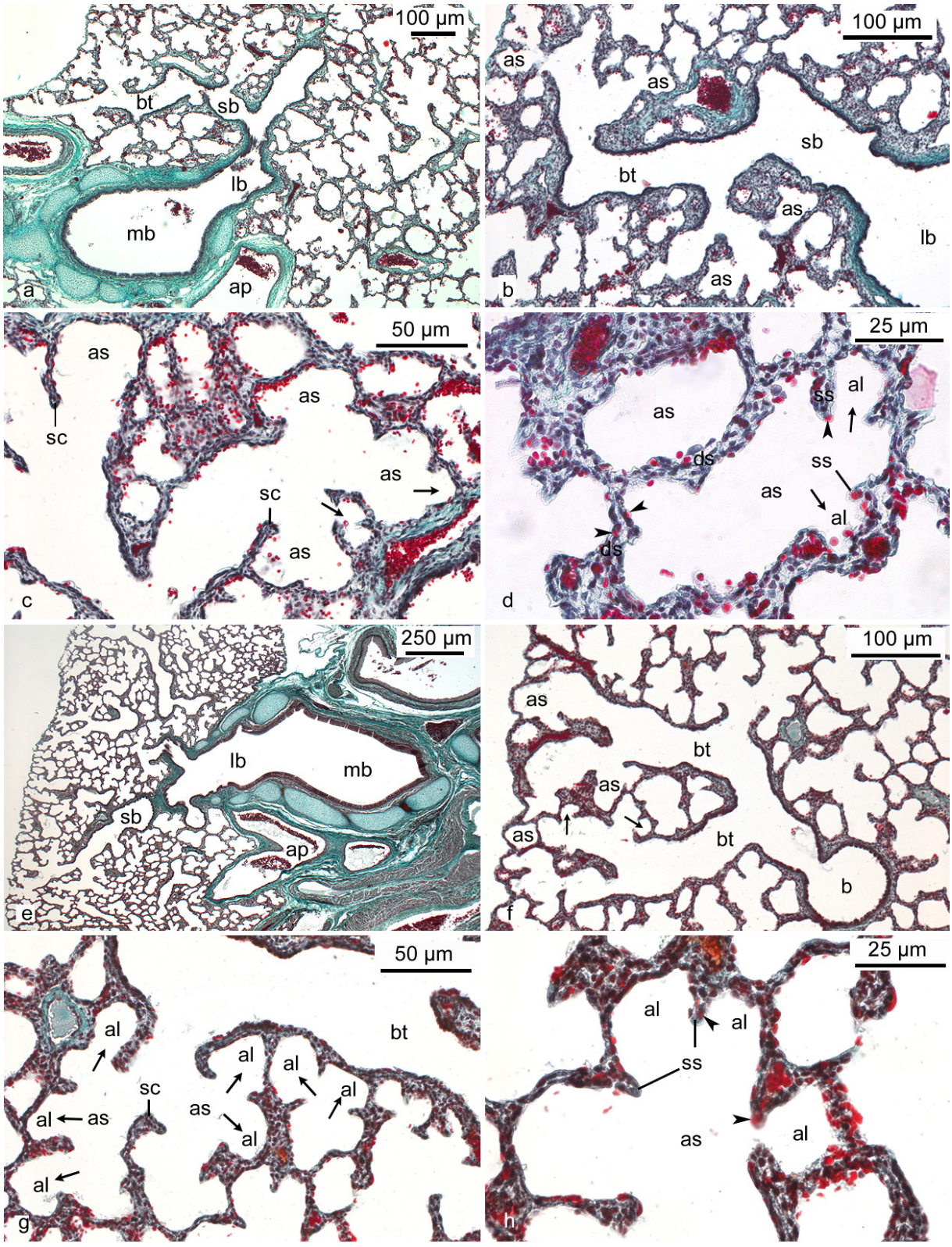

Fig. 47: Light micrographs of the 65 (a-d) and the 71 days old (e-h) lung of *Macropus eugenii*. At the age of 65 days the formation of alveoli begins. Beside air sacs of different size (c, f), first alveoli are formed by sprouting from the walls of the air sacs (c, d, g, h; alveoli indicated by arrows). The alveoli are separated from each other by single capillary septa, which protrude from the double capillary septa. Both types of septa are present at this developmental stage (d, h; capillaries are indicated by arrowheads). The bronchial tree is equivalent to that of earlier stages (a, b, e). al, alveole; ap, pulmonary artery; as, air sac; b, bronchiole; bt, terminal bronchiole; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sb, segmental bronchus; sc, septal crest; ss, single capillary septum. Trichrome staining. Magnification is indicated by the scale bar.

In the lung of the 65 days old *Macropus eugenii*, the first single capillary septa protrude from the double capillary septa and lead to the formation of the first alveoli (Fig. 47 c, d). In the new formed septa a single capillary spannes the septum. In the 65 days old pouch young, single capillary septa are still rare, but in the 71 days old *Macropus eugenii*, alveoli and single capillary septa are more frequently seen (Fig. 47 g, h). That leads to the assumption that the age of 65 days is the earliest time for the formation of alveoli.

The bronchial trees of the 65 and 71 days old lungs are widely ramified (Fig. 46 a). The main bronchi branch into lobar bronchi from which several segmental bronchi branch off (Fig. 47 a, b, e). These segmental bronchi branch into bronchioles, which give rise to terminal bronchioles in the periphery of the lung (Fig. 47 f). Compared to earlier stages of the lung development, the air compartments of the lung parenchyma have markedly increased in number and decreased in size. The branching pattern and the areas of supply of the lobar bronchi resemble that of earlier developmental stages (Fig. 46 b). With the overall size increase of the lung, the bronchial tree keeps on getting longer and the distances between the outlets of the lobar bronchi increase. In the proximal part, the main bronchi measure 500 µm in diameter. The composition of the bronchial wall is similar to that previously described. The cartilage, supporting the bronchi, extends far into distal regions of the main bronchi and is also present in the proximal parts of the lobar bronchi. The circular layer of smooth muscle cells in the wall of lobar and segmental bronchi is getting thicker and causes in the proximal parts of these bronchi longitudinal folds of the epithelium. The more distal portions of the airways, the bronchioles, are lined by cuboidal epithelium and have only a thin layer of one or two smooth muscle cells in their walls. Where the airways enter the air sacs, the cuboidal epithelium is replaced by respiratory epithelium.

The air sacs of the 65 and 71 days old lung vary in size. However, the majority of the air sacs are with approximately 50 µm in diameter small in size (Fig. 47 c, f). The septa separating the air sacs have a thickness of 5-10 µm and have still a double capillary network (Fig. 47 d).

The lung of the 98 days old *Macropus eugenii*

This stage of development is characterised by the proceeding formation of alveoli. However, there is an overlap with further tissue proliferation and terminal air sac subdivision and expansion (Fig. 48 a, b). The histological findings of the lung of the 98 days old *Macropus eugenii* are shown in figure 48.

The constituents of the bronchial tree are advanced in their structural development. Beside the cartilage in the walls of the main and lobar bronchi, a higher amount of musculature can be seen in the walls of bronchi and bronchioles. A thick layer of 3-4 smooth muscle cells leads to longitudinal folds of the epithelium even in the larger bronchioles (Fig. 48 a). The

epithelium lining the conducting airways is pseudostratified ciliated columnar epithelium in the main bronchi, simple columnar or cuboidal epithelium in the lobar and segmental bronchi and simple cuboidal epithelium in the bronchioles.

Beside small terminal air sacs of 50 µm, numerous alveoli of approximately 25 µm in diameter are present (Fig. 48 b, c, d). The single capillary septa have with 5-10 µm the same thickness as the former double capillary septa (Fig. 48 d).

The lung of the 142 days old *Macropus eugenii*

With 142 days, the air compartments of the lung parenchyma are further subdivided. They have increased in number and have decreased in size. The results of the histological investigation of the lung of the 142 days old *Macropus eugenii* are presented in figure 48. At the age of 142 days, the right lung measures 24.9 mm in total length. In relation to the CRL of approximately 122.0 mm, the right lung makes up a fifth of the body size.

The conducting airways develop to a more mature state and resemble the structure of an adult lung more closely. The cartilage, supporting the walls of the larger bronchi, extends far to the distal portions of main, lobar and even segmental bronchi (Fig. 47 e). The composition of the walls of the larger conducting airways is similar in main, lobar and segmental bronchi. The epithelium lining the main bronchi is pseudostratified ciliated columnar epithelium, whereas the lobar and segmental bronchi are lined with one-layered ciliated columnar epithelium. Beneath, a layer of a longitudinal elastic reticulum separates a thick layer of 5-10 smooth muscle cells from the epithelium. Loose connective tissue surrounds the cartilage and separates it from the muscular tissue and the lung parenchyma.

The smaller conducting airways are bronchioles. At the distal portions of the airways, they pass into terminal and respiratory bronchioles (Fig. 48 f). The epithelium lining the bronchioles is simple cuboidal epithelium, in the terminal bronchioles with cilia. A thin layer of one to two smooth muscle cells stabilises the walls of the bronchioles. Typical for respiratory bronchioles are the flattened epithelium and the alveoli at their sides.

At this developmental stage, alveoli are numerous and widespread throughout the lung. The thin walled alveoli measure 25-50 µm in diameter and radiate from alveolar sacs (Fig. 48 g). The alveoli are separated by single capillary septa, which measure 5-10 µm in thickness (Fig. 48 h). In the single capillary septum, capillaries are spanning the entire septum or occur alternately on one side and then on the other side in the septum.

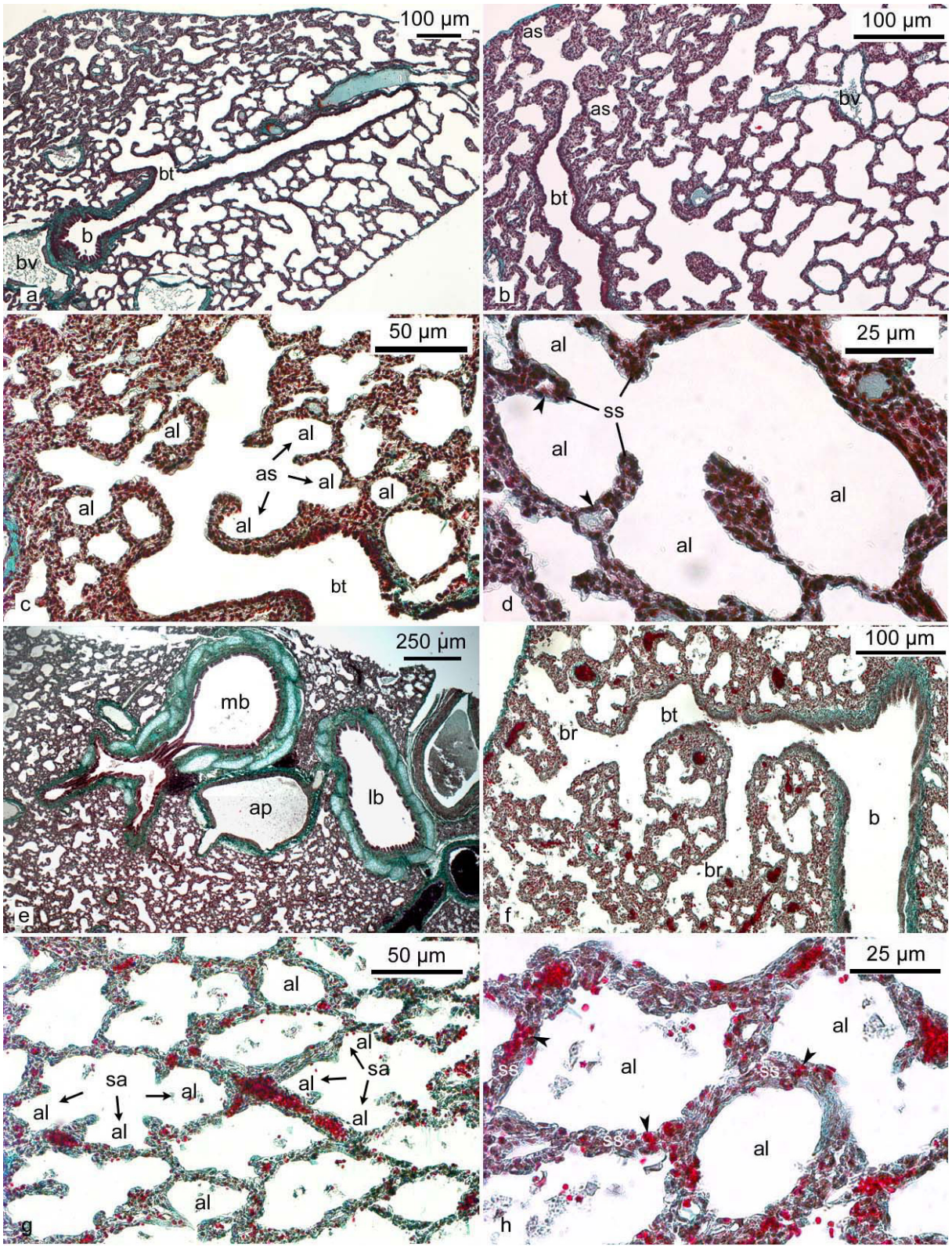

Fig. 48: Light micrographs of the 98 (a-d) and 142 days old (e-h) lung of *Macropus eugenii*. The formation of alveoli continues and is far advanced in the 142 days old lung (g). In the 98 days old lung, apart from alveoli, some air sacs are still present (a, b, c). The 142 days old lung has already alveolar sacs and respiratory bronchioles, structures typical for the adult lung (f, g; arrows indicate alveoli). Between the alveoli, septa with a single capillary bed are present (d, h, arrowheads indicate capillaries). The bronchial tree is getting larger and is supported by cartilage far to the distal portions of the airways (e). al, alveole; ap, pulmonary artery; as, air sac; b, bronchiole; br, respiratory bronchiole; bt, terminal bronchiole; lb, lobar bronchus; mb, main bronchus; sa, alveolar sac; ss, single capillary septum. Trichrome staining. Magnification is indicated by the scale bar.

3.2.2 Altricial Placentalia

At birth, the pulmonary structure depends on the extent of intra-uterine development. In altricial born mammals, such as mouse, rat, rabbit and hedgehog, the body of the newborn animal is immature in nature (Engel, 1962). So the same degree of development can be expected for the lung structure. Some investigations of the postnatal lung develop-ment in the rat (Weibel, 1967; Burri, 1974; Brody & Vaccaro, 1979; Scheuermann et al., 1988) and in the mouse (Thurlbeck, 1975; Ten Have-Opbroek, 1981) confirm this assumption.

3.2.2.1 Mesocricetus auratus

The neonate of *Mesocricetus auratus* is born at an altricial condition (see chapter 3.1.1) and is nidicolous for the first week of life. During this postnatal period, the external development (see chapter 3.1.2.3) but also the maturation of internal organs, including the lung, takes place. A survey of the structural changes during the postnatal lung development of *Mesocricetus auratus* is shown in figure 49.

The lung of the newborn *Mesocricetus auratus* is at the late terminal sac stage of lung development (Fig. 49 a). The terminal air sacs are small and numerous. At birth the conducting airways occupy a large portion of the lung volume. The ramified bronchial tree extends far to the periphery of the lung.

The postnatal lung maturation proceeds very rapid in *Mesocricetus auratus*. The first signs of the transformation from the air sac to the alveolar stage can be observed already two days after birth. The formation of alveoli is noticeable at the age of four days, but also smooth-walled air sacs are still present at this time. At this developmental stage, the lung parenchyma is highly vascularised and appears subdivided (Fig. 49 b). The lung of the seven days old *Mesocricetus auratus* exhibits some striking changes, when compared to those of the earlier stages. The formation of alveoli is advanced and becomes more evident at the age of seven days (Fig. 49 c). The previously smooth walled air sacs have now transformed into alveolar sacs, from which alveoli radiate in a concentric way. In contrast to earlier developmental stages, respiratory bronchioles and alveolar ducts can be distinguished. With 14 days the septation has made further progress and an overall size increase of the lungs took place (Fig. 49 d). The bronchial tree is widely ramified and respiratory bronchioles are common in the lung. The most striking finding of the adult lung is the overall size increase and the thinning of the septa.

The following detailed description of the lung development of *Mesocricetus auratus* is subdivided in the postnatal developmental stages examined.

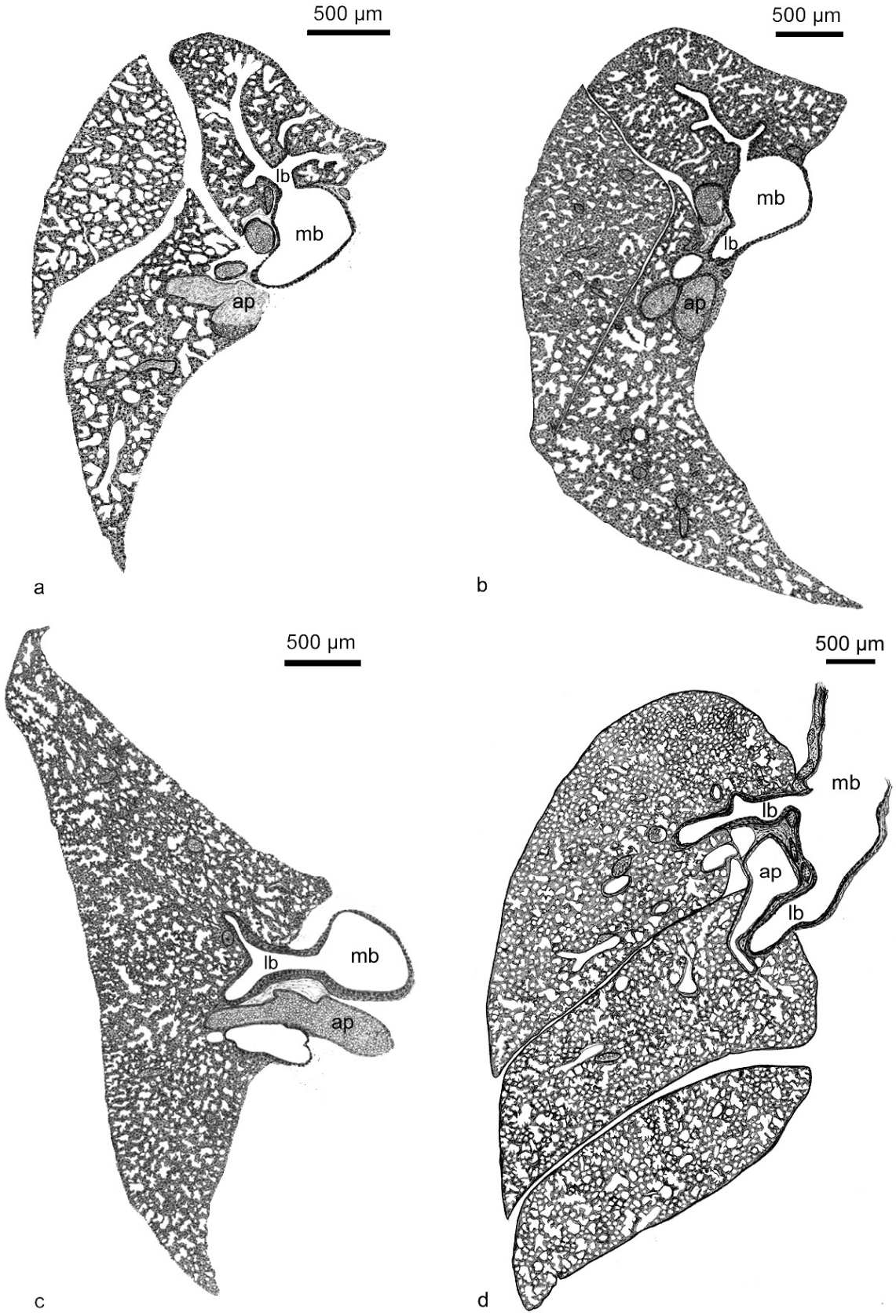

Fig. 49: Lung structure of *Mesocricetus auratus* during the postnatal development. Original drawings from histological sections of the right lung of a neonatal (a) and a 4 (b), 7 (c) and 14 days (d) old *Mesocricetus auratus.* ap, pulmonary artery; lb, lobar bronchus; mb, main bronchus. Magnification a-c 50 x, d 25 x.

The lung of the neonatal *Mesocricetus auratus*

The newborn lung of *Mesocricetus auratus* is at the late terminal sac stage of lung development. The lung parenchyma is subdivided and the numerous air sacs are small in size (Fig. 50 a, 51 a). A schematic reconstruction of the neonatal bronchial tree is shown in figure 50. The light microscopic and electron microscopic findings of the lung structure of the neonatal *Mesocricetus auratus* are presented in the figures 51 and 52.

The right lung is slightly larger than the left lung. The longitudinal expansion of the right lung is 5.0 mm, whereas the left lung measures 4.5 mm in total length. Relating to the CRL of 30 mm, the right lung takes a sixth part of the whole body length.

The right lung of *Mesocricetus auratus* is divided into superior, middle, inferior and accessory lobes. In the left lung no fissures are apparent. So the left lung has a single lobe formed by the union of the middle and inferior lobes. Only the branching pattern of the lobar bronchi indicates the several regions of the left lung (Fig. 50 b). In the right lung the superior lobe bronchus is formed by the first lateral branch from the main bronchus. The next bronchus is branching off from the lateral side of the main bronchus and supplies the right middle lobe. Approximately at the same height, the accessory lobe bronchus branches off medial from the

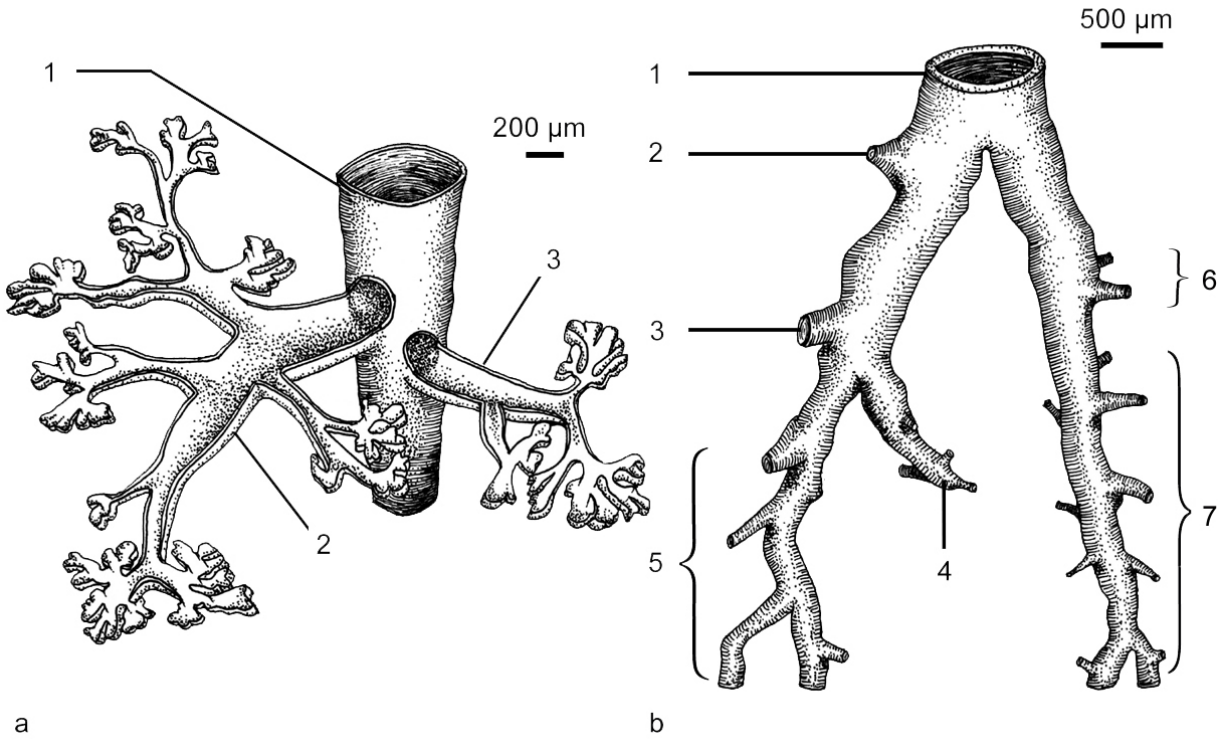

Fig. 50: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the neonatal lung of *Mesocricetus auratus*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchi; 7 – inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

main bronchus. All other branches at the distal part of the main bronchus supply the inferior lobe of the right lung. In the left lung, the superior lobe bronchus is lost. Thus two middle lobe bronchi, formed by the first branches at the dorsal and lateral side of the main bronchus,

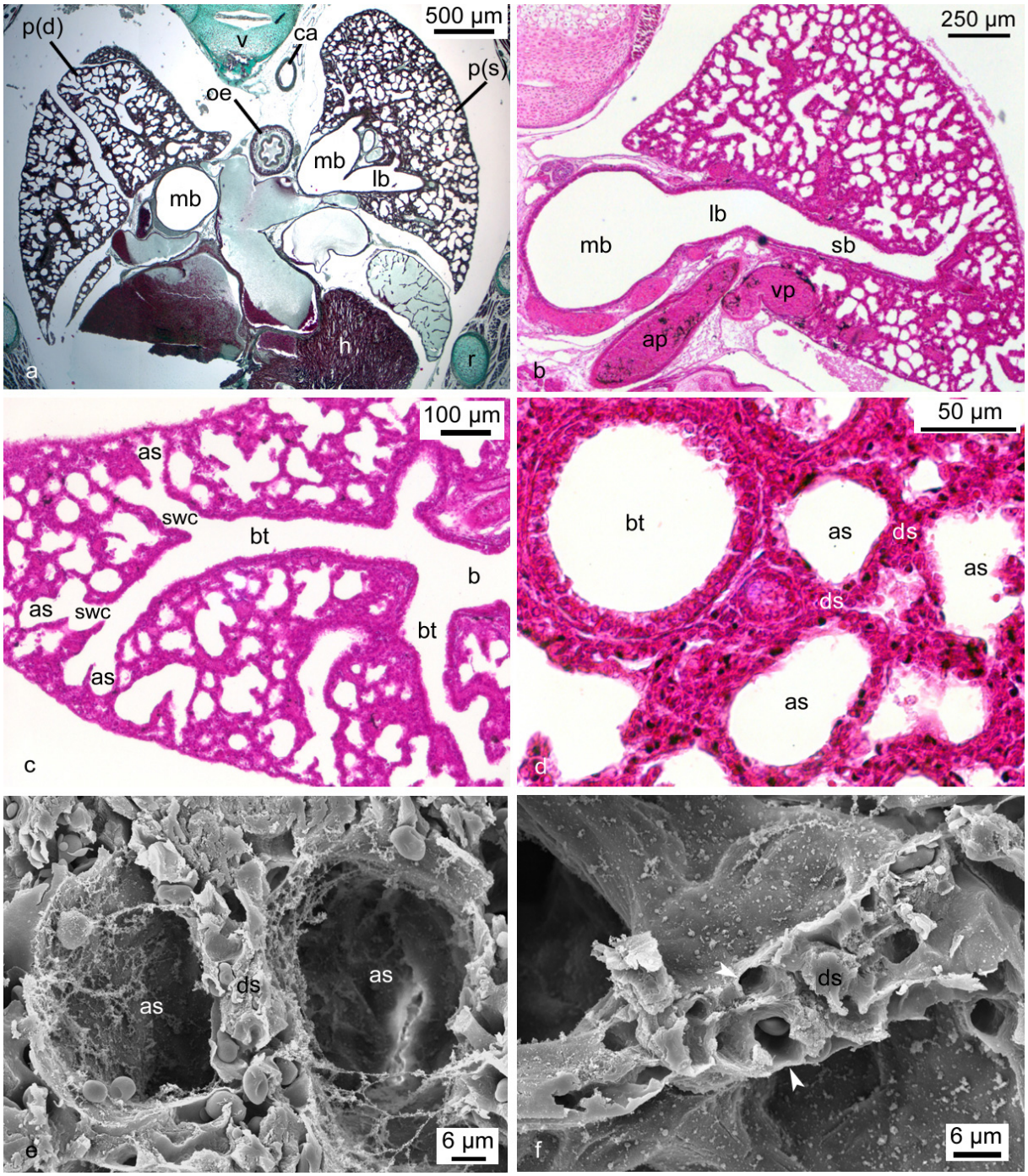

Fig. 51: Light micrographs (a-d) and scanning electron micrographs (e, f) of the lung of the neonatal *Mesocricetus auratus*. At birth the lung is at the late terminal air sac stage (a). The numerous air sacs are small in size (d, e). The ramified bronchial tree leads far into the periphery of the lung (b, c). The small air sacs are separated from each other by double capillary septa (d, e). These septa are characterised by a double capillary bed (f; capillaries are indicated by arrowheads). ap, pulmonary artery; as, air sac; b, bronchiole; bt, terminal bronchiole; ca, carotid artery; h, heart; lb, lobar bronchus; mb, main bronchus; oe, oesophagus; p(d), right lung (pulmo dextra); p(s), left lung (pulmo sinistra); ds, double capillary septum; r, rib; v, vertebra; swc, smooth-walled channel; vp, pulmonary vein. a: Trichrome staining; b-d: HE staining. Magnification is indicated by the scale bar.

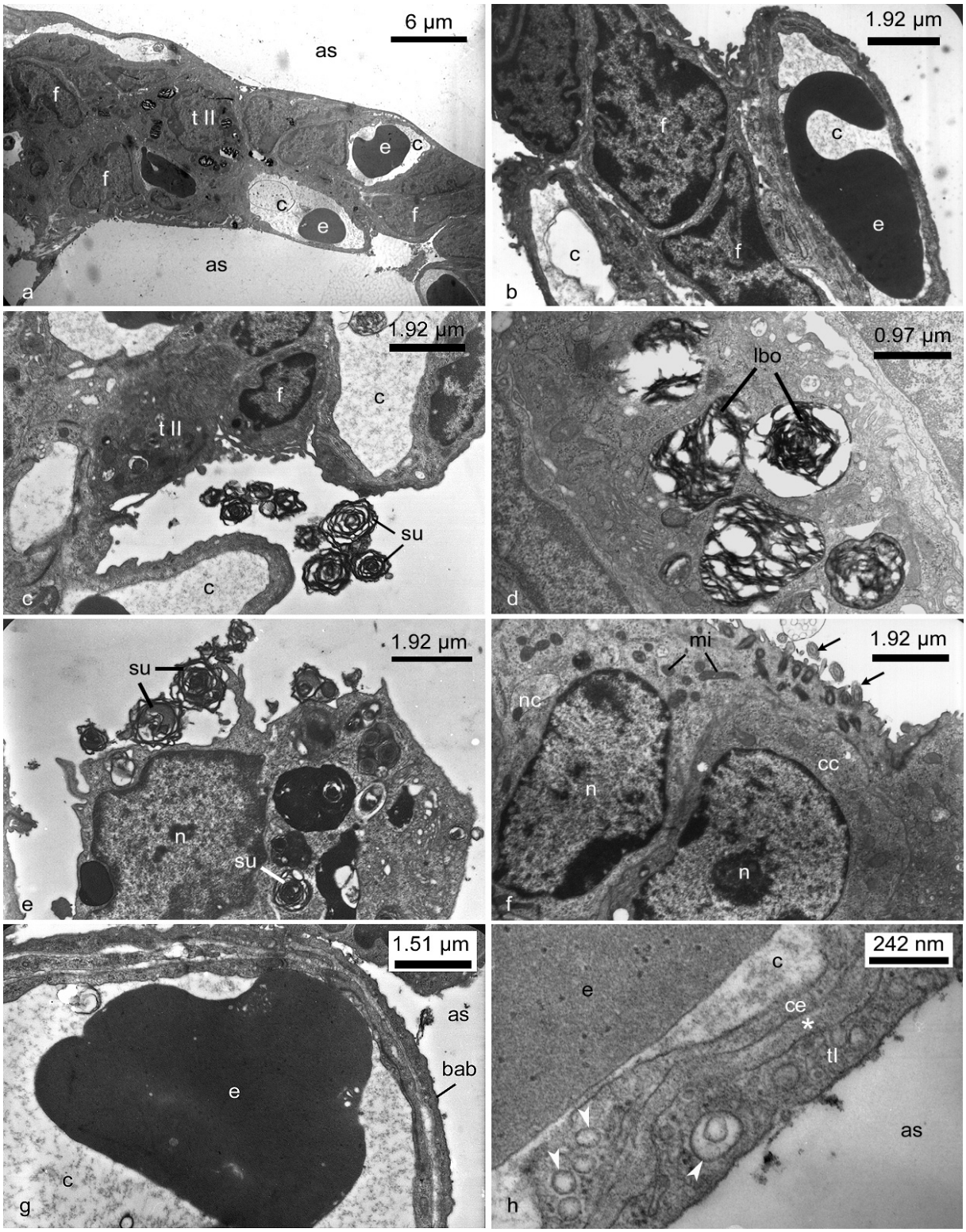

Fig. 52: Transmission electron micrographs of the newborn lung of *Mesocricetus auratus.* The septa consist of a double capillary network with a compact interstitial layer (a, b). The cuboidal type II pneumocytes produce a large quantity of phospholipids inside the lamellar bodies (c, d) and secrete it in form of surfactant (c). Surplus surfactant in the air sac lumen is removed by phagocytosis of the alveolar macrophages (e). The blood-air barrier is trilaminar in structure (capillary endothelium, epithelium and a fused basal lamina (∗) (g, h arrowheads indicate coated vesicles). The terminal bronchioles are lined by ciliated and non-ciliated cells (f, arrows indicate cilia). as, air sac; bab, blood-air barrier; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; mi, mitochondria; n, nucleus; nc, nonciliated cell; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

compensate the loss of the superior lobe bronchus. The subsequent bronchi branching off from the main bronchus supply the large inferior part of the left lung. The bronchial tree of the lung of the neonatal *Mesocricetus auratus* is well developed. In the right lung, the bronchial tree (main and lobar bronchi) shows 43 dichotomies, whereas the bronchial tree of the left lung divides less often, only 27 times.

At birth the conducting airways occupy a large portion of the lung volume. The main bronchi have a large calibre and extend through the entire lung. The system of conducting airways consisting of main, lobar and segmental bronchi and bronchioles extend far to the periphery of the lung (Fig. 51 b). The main bronchi measure 650-700 µm in diameter in the proximal part. In the more distal parts, they become smaller and measure approximately 300 µm in diameter. They are lined with one- or two-layered cuboidal ciliated epithelium, beneath a layer of three to four smooth muscle cells is lying. Loose connective tissue encloses submucosal glands and cartilage. Besides, the connective tissue separates the main bronchus from the surrounding. The cartilage extends to the second dichotomy along the main bronchial tubes and occurs only extrapulmonar. The lobar bronchi have a diameter of 150-250 µm and are lined with one-layered cuboidal ciliated epithelium. A layer of two to three smooth muscle cells is following beneath. Cartilage is missing in the walls of the lobar bronchi. The larger bronchioles measure 100 µm and the terminal bronchioles 60-80 µm in diameter. They are lined with simple cuboidal epithelium and have a circular layer of one to two smooth muscle cells in their walls. A thin layer of loose connective tissue separates the bronchioles from the surrounding lung parenchyma. In the distal portion of the terminal bronchioles ciliated and nonciliated cells can be found (Fig. 52 f). The ciliated cells have the function to move the secretions and trapped airborne particles toward the pharynx. The nonciliated or Clara cells secrete material lining the bronchiolar lumen. They have an apical region packed with granules and large mitochondria and a basal region containing the nucleus. Peripherally the epithelia of the terminal bronchioles flatten, forming straight and smooth walled channels (Fig. 51 c). These end up in the small terminal air sacs.

The terminal air sacs in the lung of the newborn *Mesocricetus auratus* are numerous and small in size (Fig. 51 d, e). They measure 50-70 µm in diameter. The air sacs are lined with respiratory epithelium, which consists mainly of squamous type I pneumocytes with occasionally interspersed type II pneumocytes. The type II pneumocytes contain lamellar bodies, which synthesise phospholipids in the form of lamellar surfactant (Fig. 52 d). The lamellar surfactant is secreted from the type II pneumocytes into the lumen of the air sacs (Fig. 52 c). In the newborn lung a lot of free surfactant can be observed. Surplus surfactant is removed by phagocytosis of alveolar macrophages, which occur free in the air space (Fig. 52 e). The double capillary septa separating the air sacs have a thickness of 10-20 µm in the newborn lung. They show little folding or branching, thus the air sacs appear smooth-walled.

The double capillary septa possess capillaries on either side, so a double capillary network is present in the neonatal lung (Fig. 51 f, 52 a, b). The septa contain a large central layer of connective tissue flanked on both sides by capillaries. The interstitial layer is rich in fibroblasts (Fig. 52 b). The erythrocytes inside of the capillaries contain no nucleus already at the time of birth (Fig. 52 g). The blood-air barrier separating air and capillary blood is trilaminar in structure. It consists of capillary endothelial cells, epithelial cells of type I pneumocytes, and a fused basal lamina of both cell types (Fig. 52 h). The diffusions barrier has a thickness of 300-400 nm and contains coated vesicles in the endothelial and the epithelial layers respectively.

The lung of the two and four days old *Mesocricetus auratus*

The lungs of the two and four day old *Mesocricetus auratus* are characterised by the beginning formation of alveoli. Whereas the appearance of the two days old lung resembles that of the newborn lung (Fig. 53 a, c), the lung of the four days old *Mesocricetus auratus* appears more subdivided (Fig. 53 e). The light microscopic and ultrastructural findings of the two and four day old lungs of *Mesocricetus auratus* are presented in the figures 53 and 54.

The bronchial tree resembles that of the neonatal lung and shows only a slight increase in length. In the four days old lung, the distal portions of the terminal bronchioles show a dense covering with ciliated cells. Compared to the newborn lung, the ciliated cells increase in number and the cilia are getting longer. In between cuboidal nonciliated cells or Clara cells can be found (Fig. 53 h).

In the two and four days old lung of Mesocricetus auratus the air sacs seem to be more irregular in shape with short ridges on the surface of the septa. Some small buds are sprouting from the air sacs and form the first alveoli (Fig. 53 b, d, f, g). The formation of alveoli seems to be a combination of several transformations. The most important aspect is the outgrowth of single capillary septa, which separate the new formed alveoli (Fig. 54 a, c). The single capillary septa protrude from the double capillary septa, which separate the air sacs. Another important process is the sprouting of the alveoli into the surrounding lung parenchyma, because this leads to an increase in the gas exchange surface area of the lung. Although the single capillary septa are formed mainly by outgrowths from the double capillary septa, there are some evidences for a transition from double capillary bed into structural single capillary septa by the fusion of capillaries (Fig. 54 b). At the age of two and four days, septa with a double capillary network are still dominating in the lung (Fig. 54 d). The double capillary septa have a thickness of 15-20 µm and a small interstitial layer between the capillary beds on both sides. The new formed single capillary septa consist of only one centrally located capillary bed abutting on both sides on the adjacent air space (Fig. 54 b).

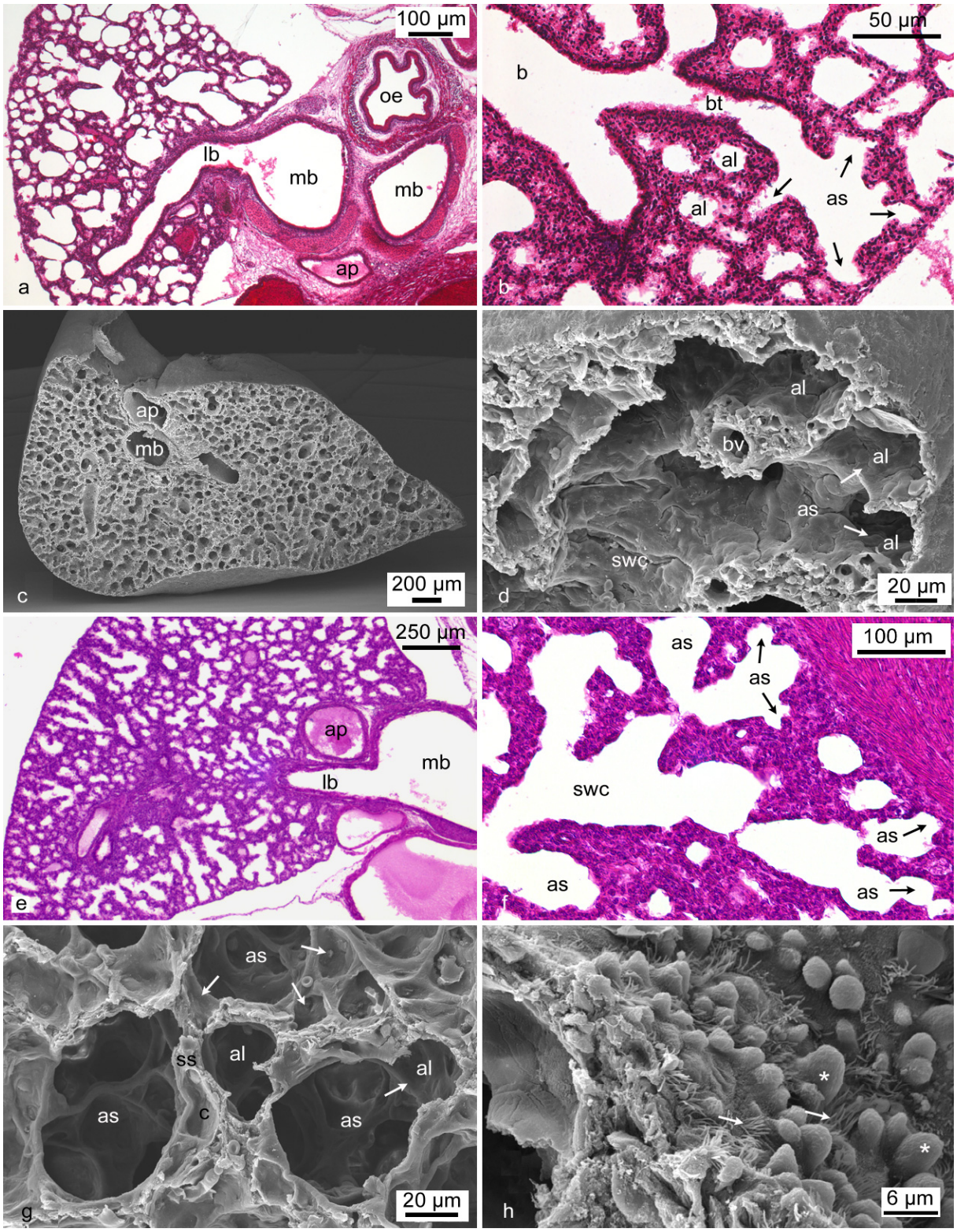

Fig. 53: Light micrographs and scanning electron micrographs of the lungs of 2 (a-d) and 4 (e-h) days old *Mesocricetus auratus*. The lung parenchyma of the 2 and 4 days old lung becomes more and more subdivided (a, c, e). That is a result of proliferation and subdivision of air sacs. Furthermore the formation of alveoli starts at the age of 2 days (b, d; alveoli are indicated by arrows) and is continued in the 4 days old lung (f, g). The alveoli are separated from each other by septa with a single capillary bed (g). In the distal portion of the terminal bronchioles ciliated and nonciliated cells are present (h, cilia indicated by arrows; nonciliated cuboidal cells marked by asterisks). al, alveole; ap, pulmonary artery; as, air sac; b, bronchiole; bt, terminal bronchiole; bv, blood vessel; c, capillary; lb, lobar bronchus; mb, main bronchus; oe, oesophagus; ss, single capillary septum; swc, smooth-walled channel; HE staining. Magnification is indicated by the scale bar.

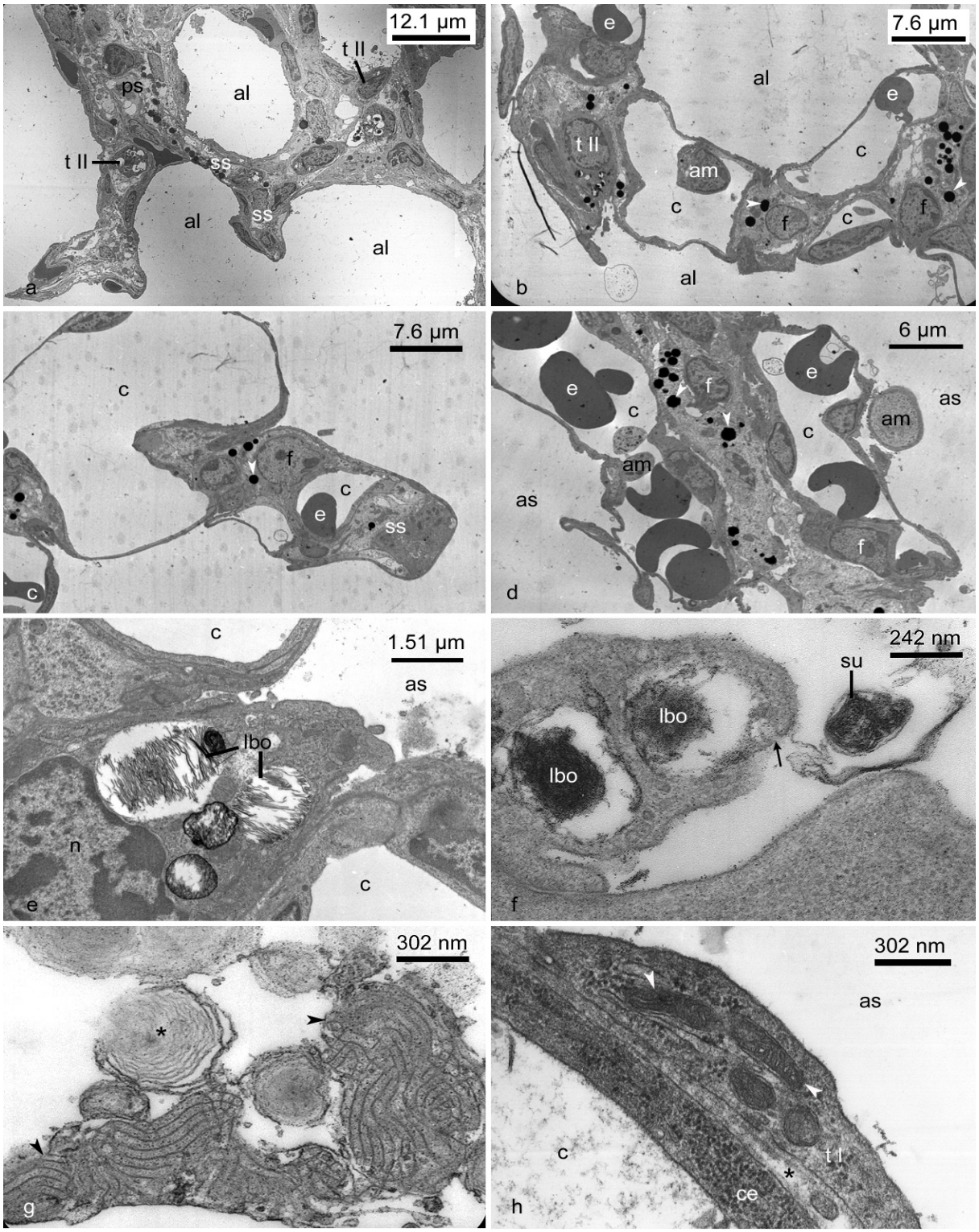

Fig. 54: Transmission electron micrographs of the lung of the 2 (b-g) and 4 (a, h) days old Mesocricetus *auratus.* Alveoli are formed by septal outgrowths (a, c). The new formed septa have a single capillary network (b, c). However, septa with a double capillary network are still present (d, fibroblasts with lipid droplets (arrowheads)). The type II pneumocytes are located at septal junctions (a). They contain lamellar bodies (e) which synthesise and secrete surfactant (f, arrow indicates exocytosis). Surfactant transforms from lamellae (∗) into a thin film (arrowheads), which covers the epithelium (g). The blood-air barrier is similar to that in the newborn lung (h, capillary endothelium, epithelium and a fused basal lamina (∗); mitochondria are indicated by arrowheads. al, alveole; am, alveolar macrophage; as, air sac; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; su, surfactant; ss, single capillary septa; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

They are thinner than the double capillary septa and measure 10-15 µm in width. The blood-air barrier is similar in structure to that in the neonatal lung (Fig. 54 h). Less attenuated areas of some type I pneumocytes within the blood-air barrier may contain mitochondria and coated vesicles.

The cuboidal type II pneumocytes are often located at septal junctions. They contain large lamellar bodies, which synthesise and store surfactant in a lamellar form (Fig. 54 e). The lamellar bodies release their contents by exocytosis (Fig. 54 f). In the air space, the lamellae unfold and transform into a thin film, which is the primary surface-active component (Fig. 54 g). The surfactant covers the air sac and alveolar epithelium and due to its biochemical properties it reduces the surface tension.

The lung of the seven days old *Mesocricetus auratus*

The lungs of the seven days old *Mesocricetus auratus* appear to be highly subdivided (Fig. 56 a, e). This results from the proceeding proliferation of lung parenchyma and the continued formation of alveoli. A schematic representation of the bronchial tree of *Mesocricetus auratus* at the age of seven days shows figure 55. The light microscopic and electron microscopic findings of the lung of a seven days old *Mesocricetus auratus* are presented in the figures 56 and 57.

The size of the right and the left lung is nearly the same. The right lung measures 5.4 mm, whereas the left lung has a total length of 5.2 mm. In relation to the body size of 45 mm, the right lung takes an eighth of the whole body length.

The branching pattern of the bronchial tree resembles that of the newborn lung (Fig. 55 b). The lobar bronchi branching off from the main bronchi have the same areas of supply. In contrast to the neonatal lung only one middle lobe bronchus can be found in the left lung. That may be a result of individual anatomical differences of the two animals examined. With the overall size increase of the lung, the bronchial tree is getting longer and the distances between the outlets of the lobar bronchi increase. The schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off shows a higher differentiated lung when compared to the neonatal lung (compare Fig. 50 a and 55 a). The bronchial tubes are getting longer and end in numerous small alveoli. The multiplicity of the small air compartments leads to an increase of the surface area of the lung. The main, lobar and segmental bronchi and the bronchioles have the same diameters and compositions of the walls as already described for the neonatal lung. There are no structural changes apparent. With the proceeding formation of alveoli, a transformation of the distal parts of terminal bronchioles takes place. New alveoli are formed also in the distal portions of

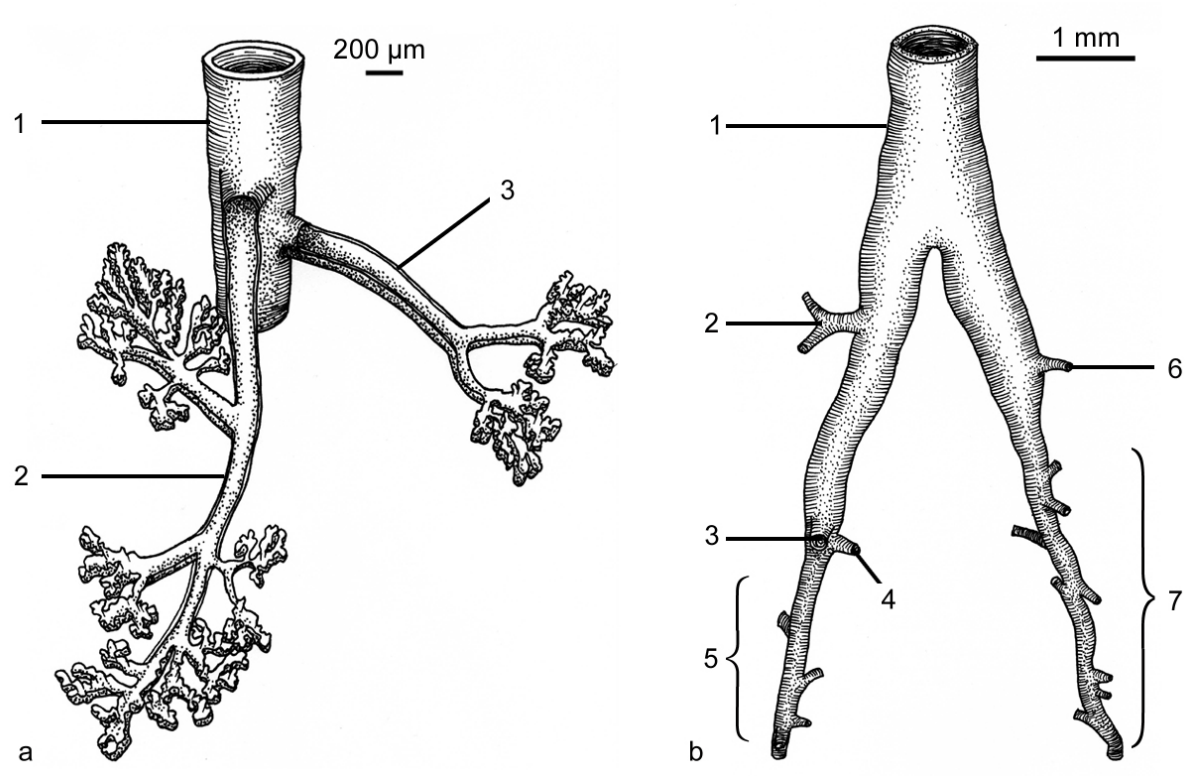

Fig. 55: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the lung of a 7 days old *Mesocricetus auratus*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 – middle lobe bronchus; 7 – inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

the bronchiolar walls. In light and scanning electron microscopy typical respiratory bronchioles with flattened epithelium and shallow depressions in their walls can be recognised (Fig. 56 b, c, f, g). These respiratory bronchioles measure 60-70 µm in diameter. Furthermore, the former smooth-walled channels transform into alveolar ducts, with alveoli at their sides (Fig. 56 c, f). They open into the former air sacs, which become now alveolar sacs with alveoli radiating in a concentric way (Fig. 56 d, h).

The alveoli measure 20-30 µm in diameter and are lined by squamous type I pneumocytes and occasionally interspersed type II pneumocytes. They are separated from each other by secondary crests, which contain a single capillary bed (Fig. 57 a). These single capillary septa have with 10-15 µm the same thickness as in the two and four days old lung of *Mesocricetus auratus* (Fig. 57 b). The interstice of the septum is relatively compact and comprises some fibroblasts with lipid droplets. The type II pneumocytes show no structural changes. They contain still many lamellar bodies (Fig. 57 c). However, less free surfactant can be observed in the alveolar lumen.

The blood-air barrier has the same structure as in the previously described developmental stages (Fig. 57 d). However, the blood-air barrier varies in thickness from 300 to 500 nm.

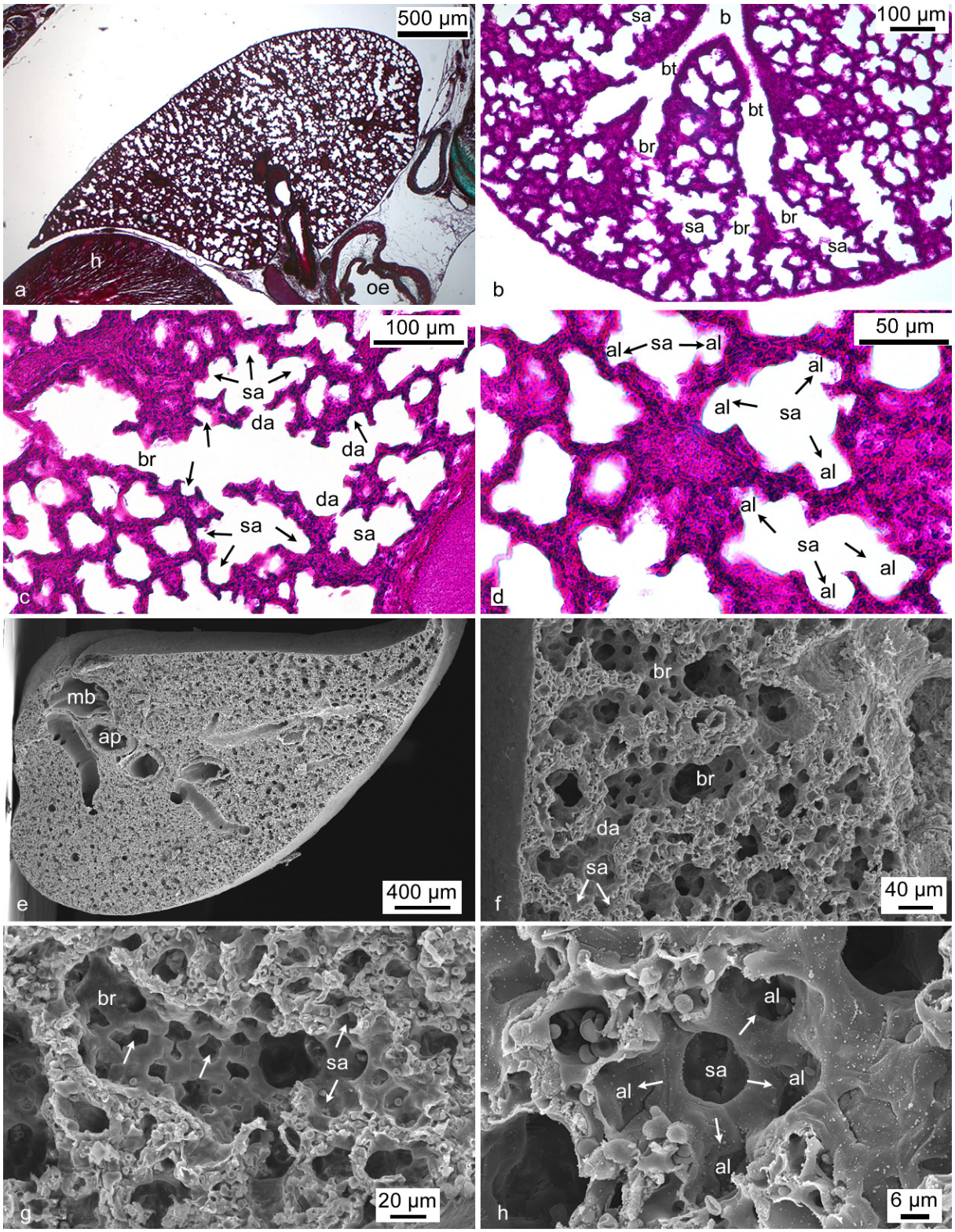

Fig. 56: Light micrographs and scanning electron micrographs of the lung of the 7 days old *Mesocricetus auratus*. Compared to earlier stages, the lung parenchyma of the 7 days old lung appears highly subdivided (a, e). This is resulting from the proceeding proliferation of lung parenchyma and the continued formation of alveoli. (c, d; alveoli are indicated by arrows). With the formation of alveoli new structures in the terminal airways develop. The distal portion of terminal bronchioles transform into respiratory bronchioles, with alveoli at their sides (b, f, g). The short afferent smooth-walled channels transform into alveolar ducts and open into alveolar sacs, from which alveoli radiate in a concentric way (c, d, h). al, alveole; ap, pulmonary artery; b, bronchiole; bt, terminal bronchiole; br, respiratory bronchiole; da, alveolar duct; h, heart; mb, main bronchus; oe, oesophagus; sa, alveolar sac. a: Trichrome; b: HE staining. Magnification is indicated by the scale bar.

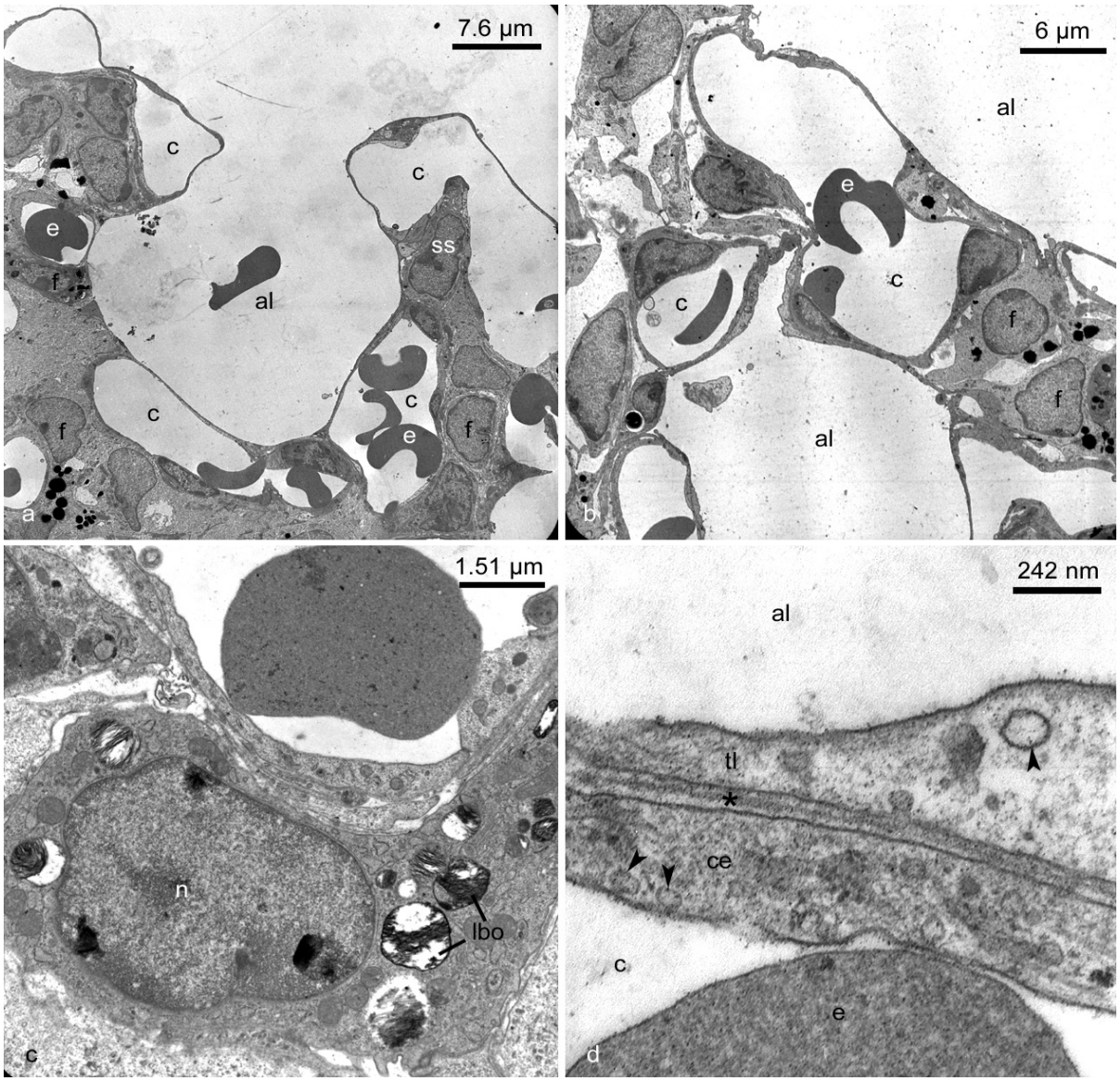

Fig. 57: Transmission electron micrographs of the lung of the 7 days old Mesocricetus *auratus.* Alveoli are separated from each other by septa with a single capillary bed (a, b). Compared to earlier stages, the type II pneumocytes show no structural changes (c). They contain phospholipids inside the lamellar bodies. The blood-air barrier is trilaminar and consists of capillary endothelial cells, type I pneumocytes and a fused basal lamina (∗) of both cell types; coated vesicles are indicated by arrowheads. al, alveole; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; ss, single capillary septa; t I, type I pneumocyte. Magnification is indicated by the scale bar.

Some coated vesicles are present in the epithelial and endothelial cells of the diffusions barrier. They may be involved in the transport of proteins or in the removal of small air borne particles.

The lung of the 11 and 14 days old *Mesocricetus auratus*

At these developmental stages the septation of the lung parenchyma has made further progress (Fig. 58 a, e). The histological findings of the lung structure of the 11 and 14 days

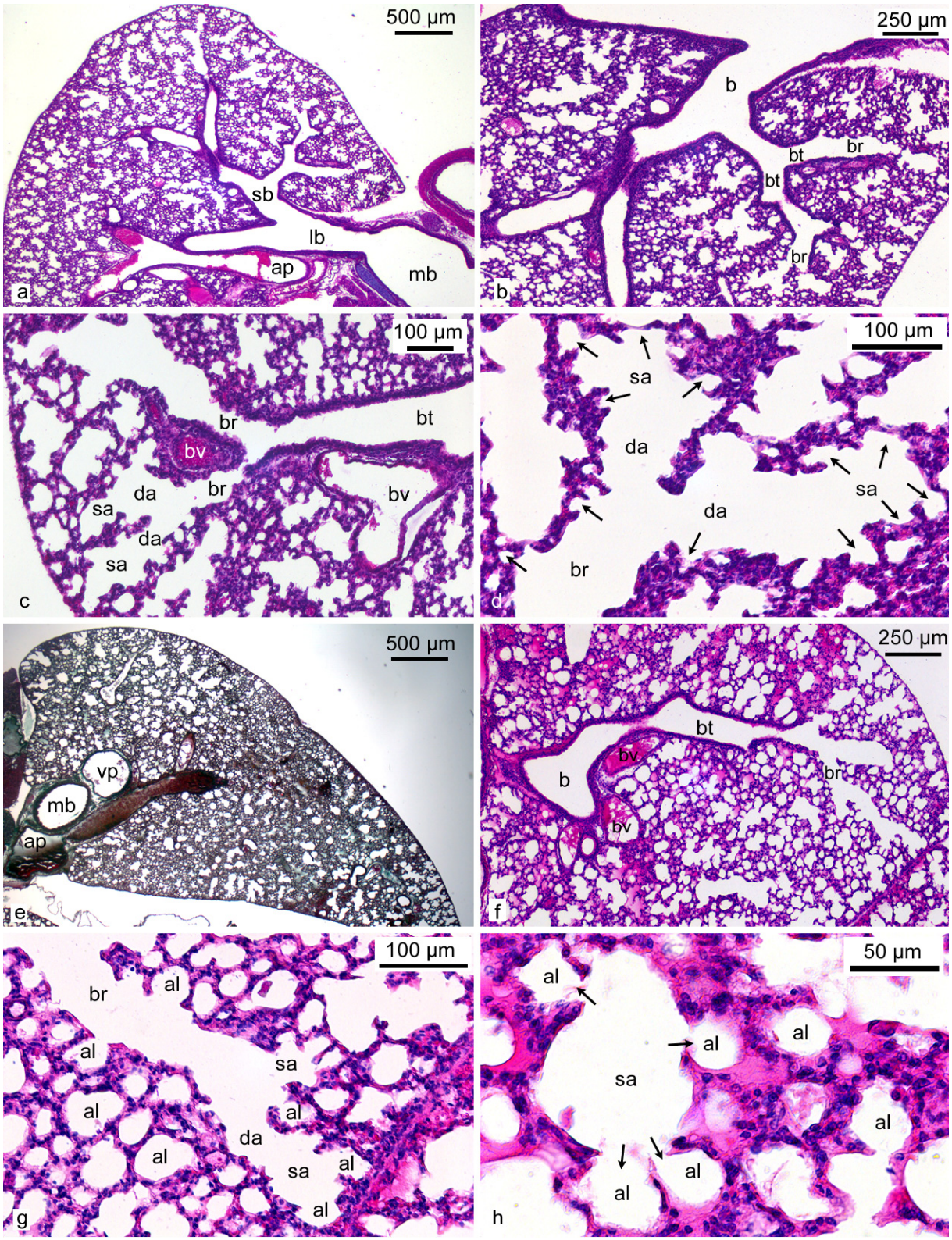

Fig. 58: Light micrographs of the lungs of the 11 (a-d) and 14 (e-h) days old *Mesocricetus auratus*. At these developmental stages the septation of the lung parenchyma has made further progress (a, e). The bronchial tree is well developed and leads with many dichotomies deep into the periphery of the lung (a, b, f). In the terminal airways respiratory bronchioles and alveolar ducts are common (c, d, f, g). The alveolar ducts open into alveolar sacs, from which alveoli radiate in a concentric way (d, h; alveoli are indicated by arrows). al, alveole; ap, pulmonary artery; b, bronchiole; bt, terminal bronchiole; br, respiratory bronchiole; bv, blood vessel; da, alveolar duct; lb, lobar bronchus; mb, main bronchus; sa, alveolar sac; sb, segmental bronchus; vp, pulmonary vein. e: Trichrome; a-d, f-h: HE staining. Magnification is indicated by the scale bar.

old *Mesocricetus auratus* are presented in figure 58.

Compared to the lung at the age of seven days, at day 14 a clear size increase of the lungs took place. The right lung measures 12.3 mm in total length, whereas the left lung is slightly smaller with an overall size of 11.1 mm.

The bronchial tree appears to be consistent in structure and diameter of the airways and resembles that described for the newborn *Mesocricetus auratus*. However, the bronchial and bronchiolar tubes have to increase in length in order to supply the peripheral regions of the lung (Fig. 58 a, b, f).

Further new alveoli are formed in the distal parts of the bronchiolar walls, so that the respiratory bronchioles become more numerous and increase in length. The respiratory bronchioles pass into shorter alveolar ducts, which open immediately into alveolar sacs (Fig. 58 c, d, g).

From the alveolar sacs the alveoli radiate and sprout into the surrounding parenchyma (Fig. 58 h). The formation of alveoli proceeds, what leads to an increase in the number of alveoli. Additional single capillary septa have appeared so that different stages of septal development can be seen. The older septal crests have grown in length, whereas new septa protruding from older septa are short.

The lung of the adult *Mesocricetus auratus*

The overall appearance of the lung parenchyma of the adult *Mesocricetus auratus* is highly subdivided (Fig. 59 a). The striking findings of the adult lung are the enormous thinning of the septa and of the blood-air barrier. The light microscopic and electron microscopic findings of the adult lung of *Mesocricetus auratus* are presented in the figures 59 and 60. The bronchial tree is getting longer in consequence of the overall size increase of the lung. However, the structure of the bronchial and bronchiolar walls remains constant on the whole. The main bronchi measure 750 µm in diameter in the proximal part and approximately 350 µm in the more distal parts. They are lined by one- or two-layered cuboidal ciliated epithelium. Beneath, a thick layer of three to four smooth muscle cells stabilise the bronchial wall. Cartilage is present only in the extrapulmonar part of the main bronchus, until the bronchus enters the lung in the hilar region. The walls of the lobar and segmental bronchi are lined by one- or two-layered cuboidal epithelium and are with a layer of two to three smooth muscle cells less muscular.

The bronchioles of the adult lung are lined by a simple cuboidal epithelium and their walls consist of a single layer of smooth muscle cells. The terminal bronchioles measure 50-60 µm in diameter (Fig. 59 b, e). Electron micrographs reveal a relatively constant ratio of ciliated

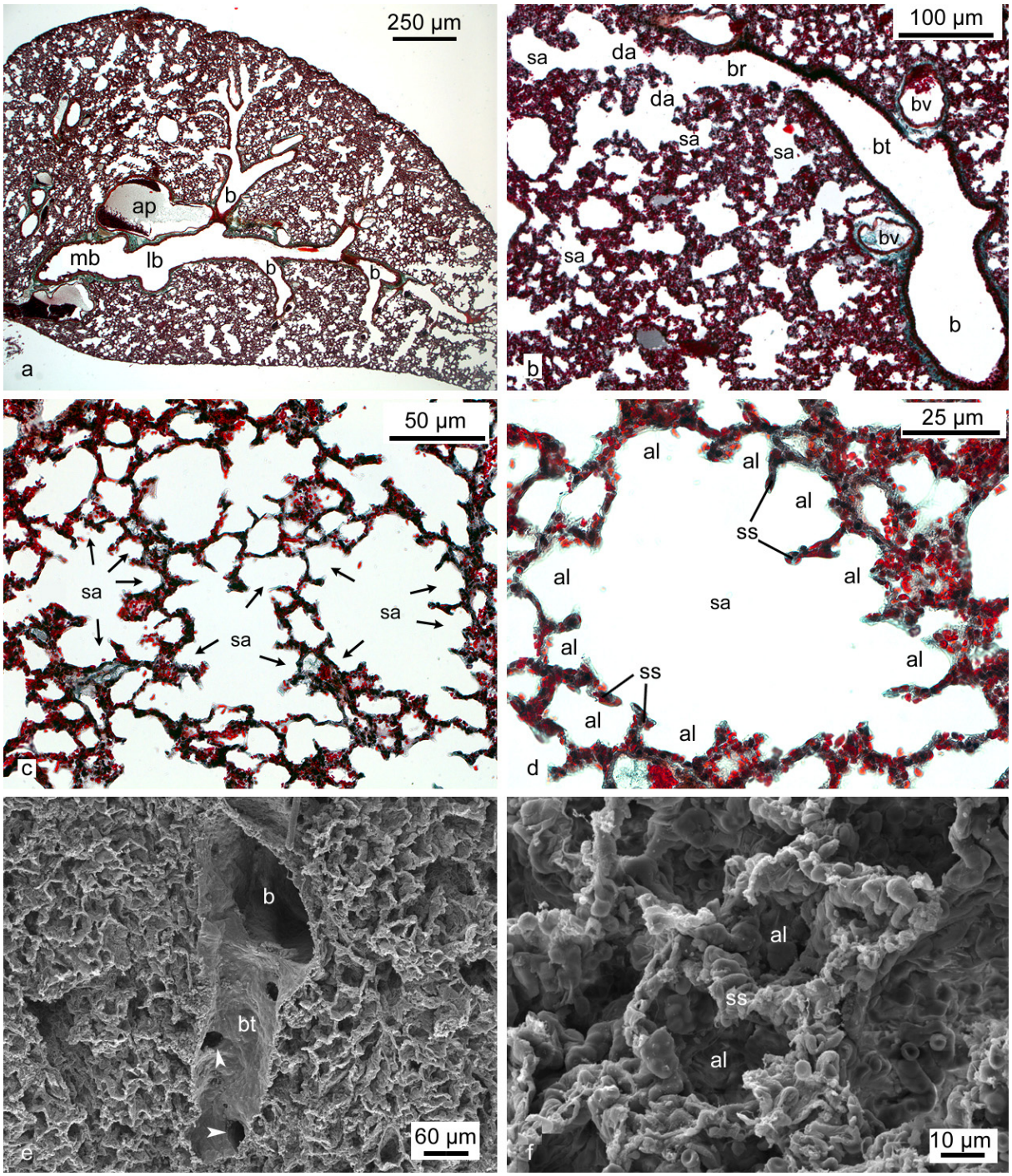

Fig. 59: Light micrographs and scanning electron micrographs of the adult lung of *Mesocricetus auratus*. The lung parenchyma of the adult lung is highly subdivided and the bronchial tree is widely ramified to reach also the most peripheral regions of the lung (a). The most distal parts of the airways consist of terminal bronchioles, respiratory bronchioles, alveolar ducts and alveolar sacs (b, e; arrowheads indicate outlets of bronchioles). The alveolar sacs have a big lumen and numerous small alveoli radiate in the surrounding parenchyma (c, d; alveoli are indicated by arrows). The alveoli are separated by thin single capillary septa which grow out from older septa (d, f). al, alveole; ap, pulmonary artery; b, bronchiole; bt, terminal bronchiole; br, respiratory bronchiole; bv, blood vessel; da, alveolar duct; lb, lobar bronchus; mb, main bronchus; sa, alveolar sac; ss, single capillary septum. Trichrome staining. Magnification is indicated by the scale bar.

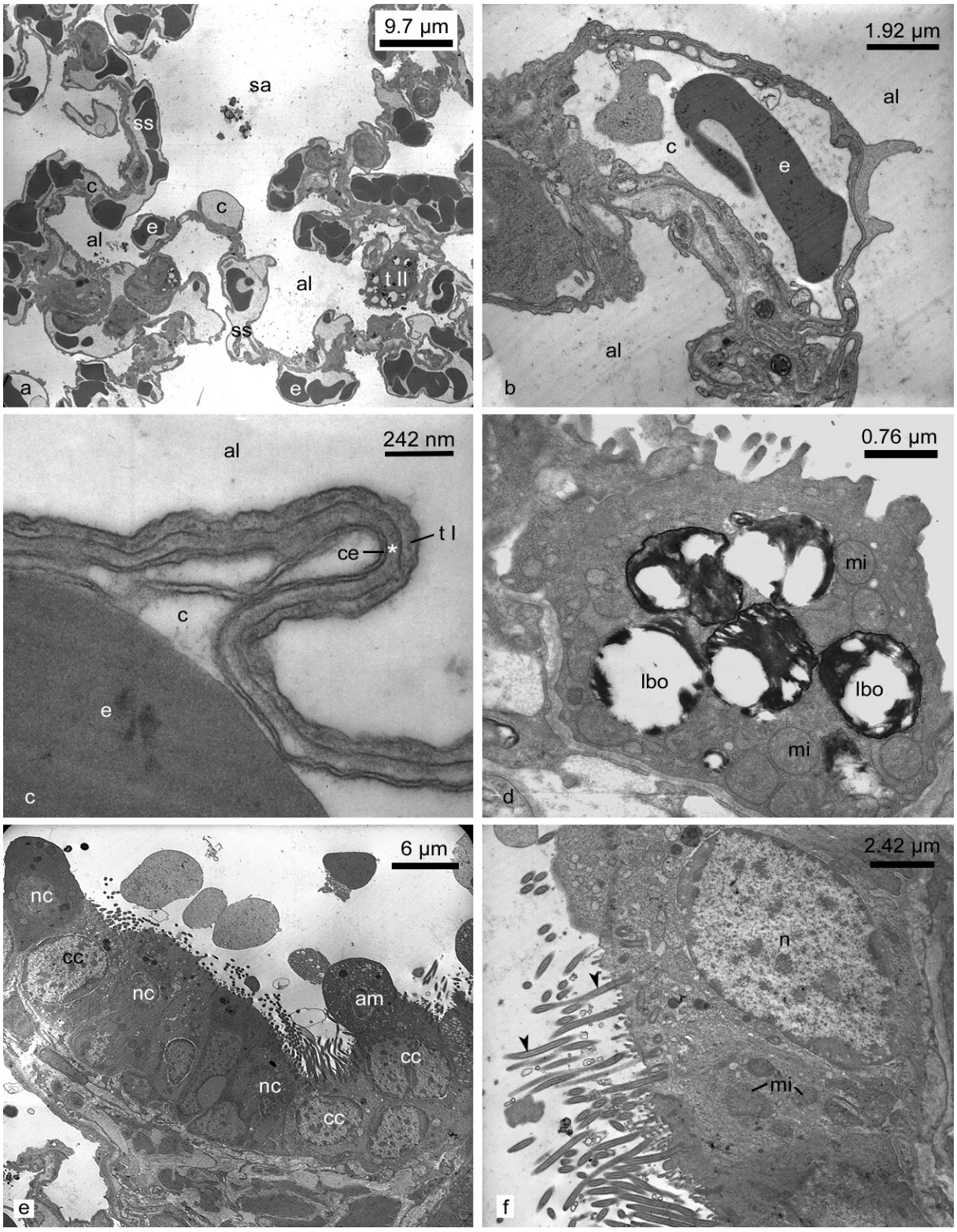

Fig. 60: Transmission electron micrographs of the adult lung of *Mesocricetus auratus.* Alveoli radiate from alveolar sacs and are separated by thin septa with a single capillary bed (a, b). The blood-air barrier becomes progressively thinner with age. However, it remains constant in the trilaminar structure consisting of capillary endothelial cells, type I pneumocytes and a fused basal lamina (∗) (c). The type II pneumocytes contain lamellar bodies and mitochondria (d). The distal portions of terminal bronchioles are lined by ciliated and nonciliated cells (e). The ciliated cells have long cilia (arrowheads) and mitochondria in their apical region and a nucleus in the basal region. al, alveole; am, alveolar macrophage; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; lbo, lamellar body; mi, mitochondria; n, nucleus; nc, nonciliated cell; sa, alveolar sac; ss, single capillary septum; t I, type I pneumocyte; t II pneumocyte. Magnification is indicated by the scale bar.

and nonciliated cells (Fig. 60 e). The free surfaces of nonciliated cells bulge into the bronchiolar lumen, whereas the ciliated cells protrude into the lumen only with their long cilia (Fig. 60 f). The terminal bronchioles give rise to alveolarised respiratory bronchioles, which are smaller and measure only 30-40 µm in diameter. The respiratory bronchioles pass into smaller alveolar ducts, which are characterised by a high degree of alveolarisation (Fig. 59 b). The alveolar ducts open into large alveolar sacs, which measure 50-80 µm in diameter (Fig. 59 c, d). From the centres of the alveolar sacs numerous small alveoli radiate.

The alveoli become slightly smaller and measure 20-25 µm in diameter (Fig. 59 d, f). But due to the proceeding formation of alveoli the number of alveoli has remarkably increased. Type I and type II pneumocytes can be distinguished lining the alveolar region. The type I pneumocytes cover most of the alveolar surface with long, thin extensions. The type II pneumocytes are large cuboidal cells, exposing microvilli on the free luminal surface. In the cytoplasm osmiophilic, lamellar bodies are typical (Fig. 60 d). Furthermore mitochondria and endoplasmic reticulum can be distinguished. The single capillary septa separating the alveoli become thinner. They measure now 5-7 µm in width. The capillaries are situated in the centre of the septum or alternately protruding at one side of a thin axis of connective tissue components. They form a single capillary network (Fig. 60 a, b). The interstitial compartment is markedly reduced, the fibroblasts appear to be less numerous and smaller. Just as the septa, also the blood-air barrier becomes thinner. The diffusion barrier has a thickness of 130-180 nm. However, the trilaminar structure is retained. It consists of capillary endothelium, alveolar epithelium and a fused basal lamina of both cell types. In particular the endothelial layer is extremely thin. In addition the surface area of the diffusion barrier is increased by the formation of folds (Fig. 60 c).

3.2.2.2 Suncus murinus

The neonate of *Suncus murinus* is born in an altricial condition (see chapter 3.1.1). In the first week of life the young is lying in the nest. During this short postnatal period it passes through a rapid development of the external characters (see chapter 3.1.2.4). However, also the internal organs, including the lung, mature during this time. A survey of the structural changes during the postnatal lung development of *Suncus murinus* is shown in figure 61.

The lung of the newborn *Suncus murinus* is at the late terminal sac stage of lung development (Fig. 61 a). The terminal air sacs are small and numerous. The well developed bronchial tree is ramified and extends to the periphery of the lung. In *Suncus murinus* the postnatal lung maturation proceeds fast. The first formation of alveoli is noticeable at the age of four days. But at this time the smooth-walled air sacs are still present and form the majority of the air compartments in the lung (Fig. 61 b).

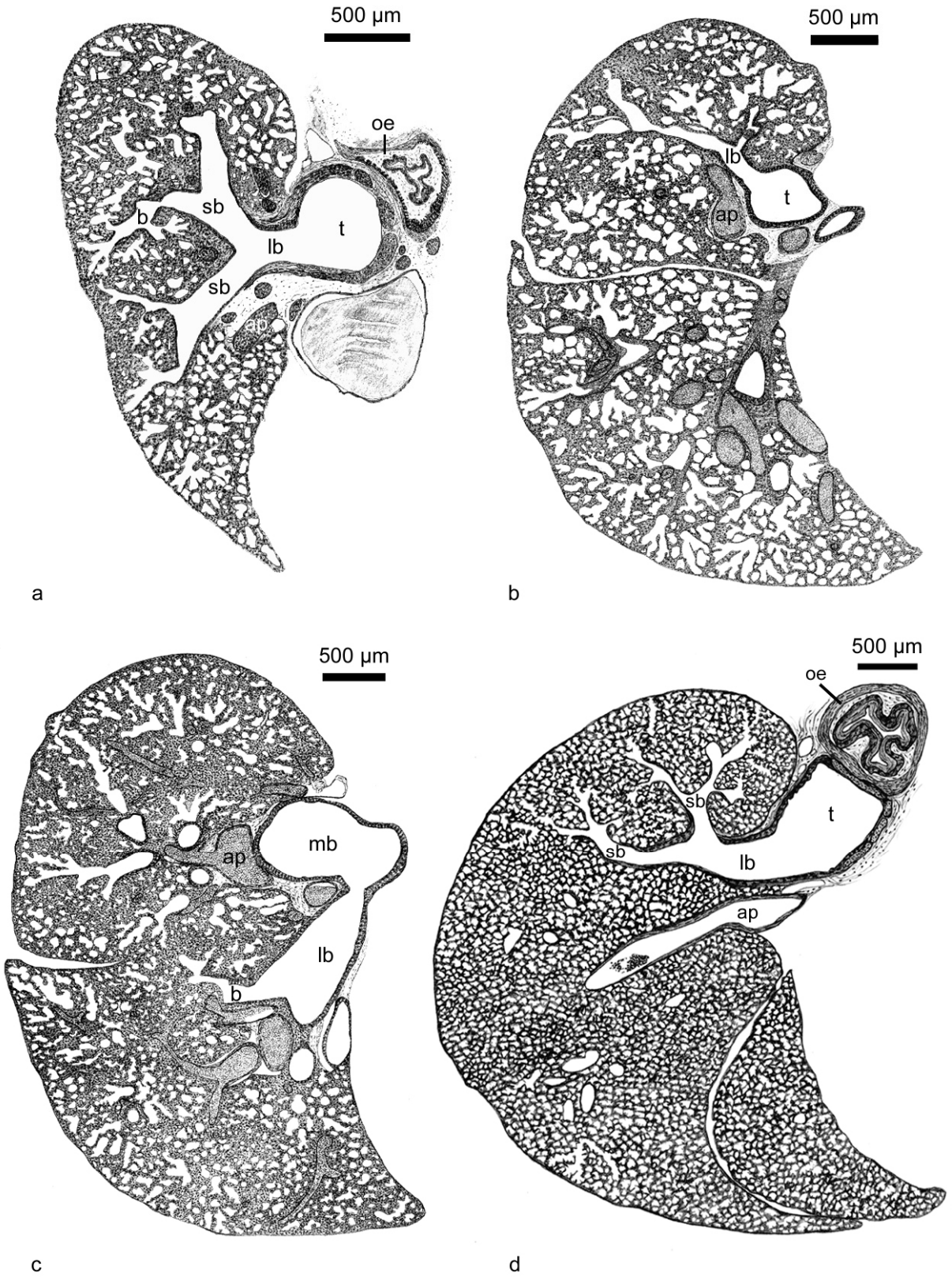

Fig. 61: Lung structure of *Suncus murinus* during the postnatal development. Original drawings from histological sections of the right lung of a neonate (a) and a 4 (b), 7 (c) and 14 days (d) old *Suncus murinus.* ap, pulmonary artery; b, bronchiole; lb, lobar bronchus; mb, main bronchus; oe, oesophagus; sb, segmental bronchus; t, trachea. Magnification a-c 50 x, d 25 x.

In the lung of the seven days old *Suncus murinus* the formation of alveoli is advanced and becomes more evident (Fig. 61 c). With the proceeding formation of alveoli several transformations took place in the lung. The previously smooth walled air sacs have now transformed into alveolar sacs, from which alveoli radiate in a concentric way. Furthermore, new alveoli are also formed in the distal parts of the bronchiolar walls. That leads to the formation of respiratory bronchioles and alveolar ducts in the terminal airways. With 14 days the septation has made further progress and the lung appears highly sudivided (Fig. 61 d). A marked increase of the surface area of the lung can be observed. The bronchial tree is widely ramified and extended. Alveolar sacs, alveolar ducts and respiratory bronchioles are common in the lung. The lung of the adult *Suncus murinus* is characterised by an overall size increase and the thinning of the septa.

The following detailed description of the lung development of *Suncus murinus* is subdivided in the postnatal developmental stages examined.

The lung of the neonatal *Suncus murinus*

At birth the lung of *Suncus murinus* is at the late terminal sac stage of lung development. The small terminal air sacs are numerous and give the lung parenchyma a subdivided appearance (Fig. 63 a, e). A schematic reconstruction of the neonatal bronchial tree is shown in figure 62. The light microscopic and electron microscopic findings of the lung structure of the neonatal *Suncus murinus* are presented in the figures 63 and 64.

The right lung is noticeable larger than the left lung. The longitudinal expansion of the right lung is 4.7 mm, whereas the left lung measures 3.5 mm in total length. Relating to the CRL of 35 mm, the right lung takes a seventh part of the whole body length.The right lung of *Suncus murinus* is divided into superior, middle, inferior and accessory lobes. The left lung shows no fissures. So the left lung has a small single lobe, consisting only of the inferior lobe. The pulmonary lobes are indicated by the branching of the respective lobe bronchi (Fig. 62 b). In the right lung, the superior lobe has a tracheal bronchus, which arises directly from the right side of the trachea. It is subdivided into cranial and caudal branches. The middle lobe bronchus is the first branch of the lateral side of the right main bronchus. Beneath, the accessory lobe bronchus branches off medial from the main bronchus. All other branches at the distal part of the main bronchus supply the inferior lobe of the right lung. The left main bronchus is extremely slender, as compared to the right main bronchus. That may be result from the absence of superior and middle lobe bronchi. The remaining bronchi branching off from the main bronchus supply the single inferior lobe of the left lung. The bronchial tree of the right lung is well developed.

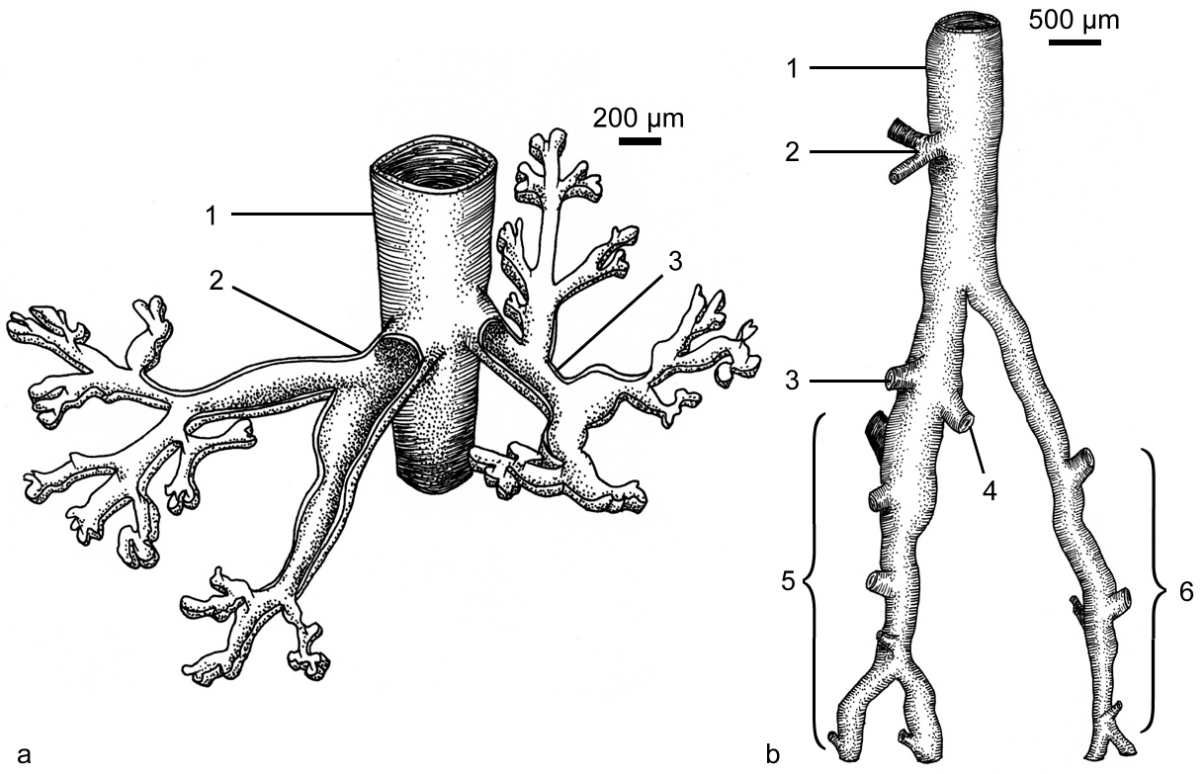

Fig. 62: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the lung of the neonatal *Suncus murinus*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 - inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

Alltogether 45 dichotomies of the larger bronchi (main and lobar bronchi) may be counted. The bronchial tree of the smaller left lung divides less often, only 16 times. In the lung of the neonatal *Suncus murinus* the system of conducting airways, consisting of main, lobar and segmental bronchi and bronchioles, is well developed. The conducting airways occupy a large portion of the lung volume and extend far into the periphery of the lung (Fig. 63 b, c). A peculiarity of the *Suncus murinus* lung is the branching of the right superior lobe bronchus directly from the trachea. The trachea measures 650-700 µm in diameter and is lined with one-layered cuboidal or columnar ciliated epithelium. Beneath, a thick layer of four to five smooth muscle cells is lying. Where cartilage supports the tracheal wall, the muscular layer is thinner, only two to three smooth muscle cells. For stabilisation the trachea is surrounded by cartilage, which is intermitted only at the point where the superior lobe bronchus is branching off. The cartilage is surrounded by loose connective tissue, which encloses also submucosal glands and separates the trachea from the surrounding. Short after the bifurcation the right main bronchus measures 600-650 µm in diameter, whereas the left main bronchus is smaller with 500 µm in diameter. In the more distal parts, both main bronchi become smaller and measure 300-350 µm in diameter in the right lung and approximately

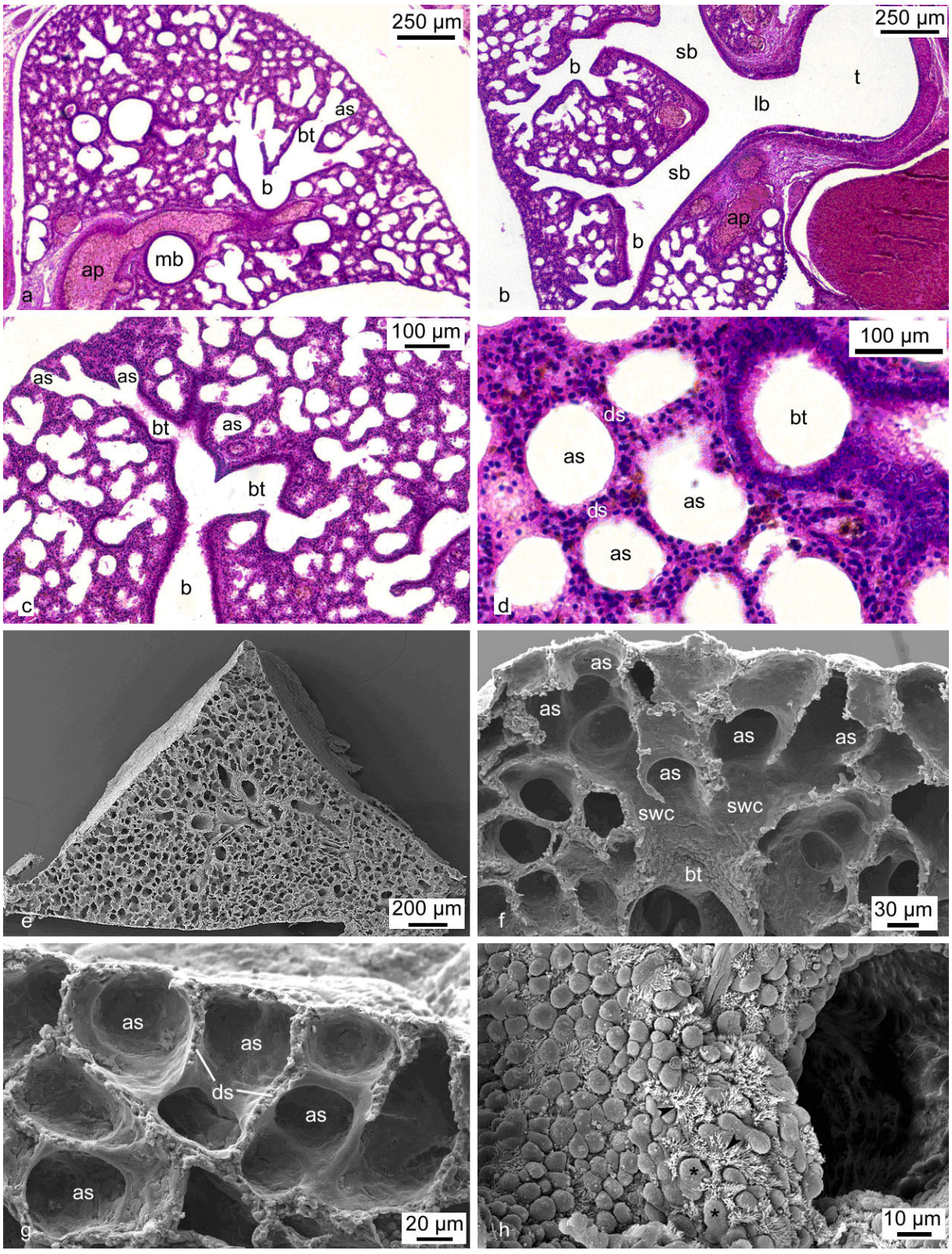

Fig. 63: Light micrographs (a-d) and scanning electron micrographs (e, f) of the lung of the neonatal *Suncus murinus*. At birth the lung is at the terminal air sac stage (a, b). The numerous air sacs are small in size (d, g). The bronchial tree is ramified and extends to the periphery of the lung (b, c, f). The small air sacs are separated from each other by double capillary septa (d, g). In the distal portion of the terminal bronchioles short cilia indicate the beginning covering with cilia (h, asterisk indicate nonciliated cells, arrowheads indicate cilia). ap, pulmonary artery; as, air sac; b, bronchiole; bt, terminal bronchiole; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; sb, segmental bronchus; swc, smooth-walled channel; t, trachea; HE staining. Magnification is indicated by the scale bar.

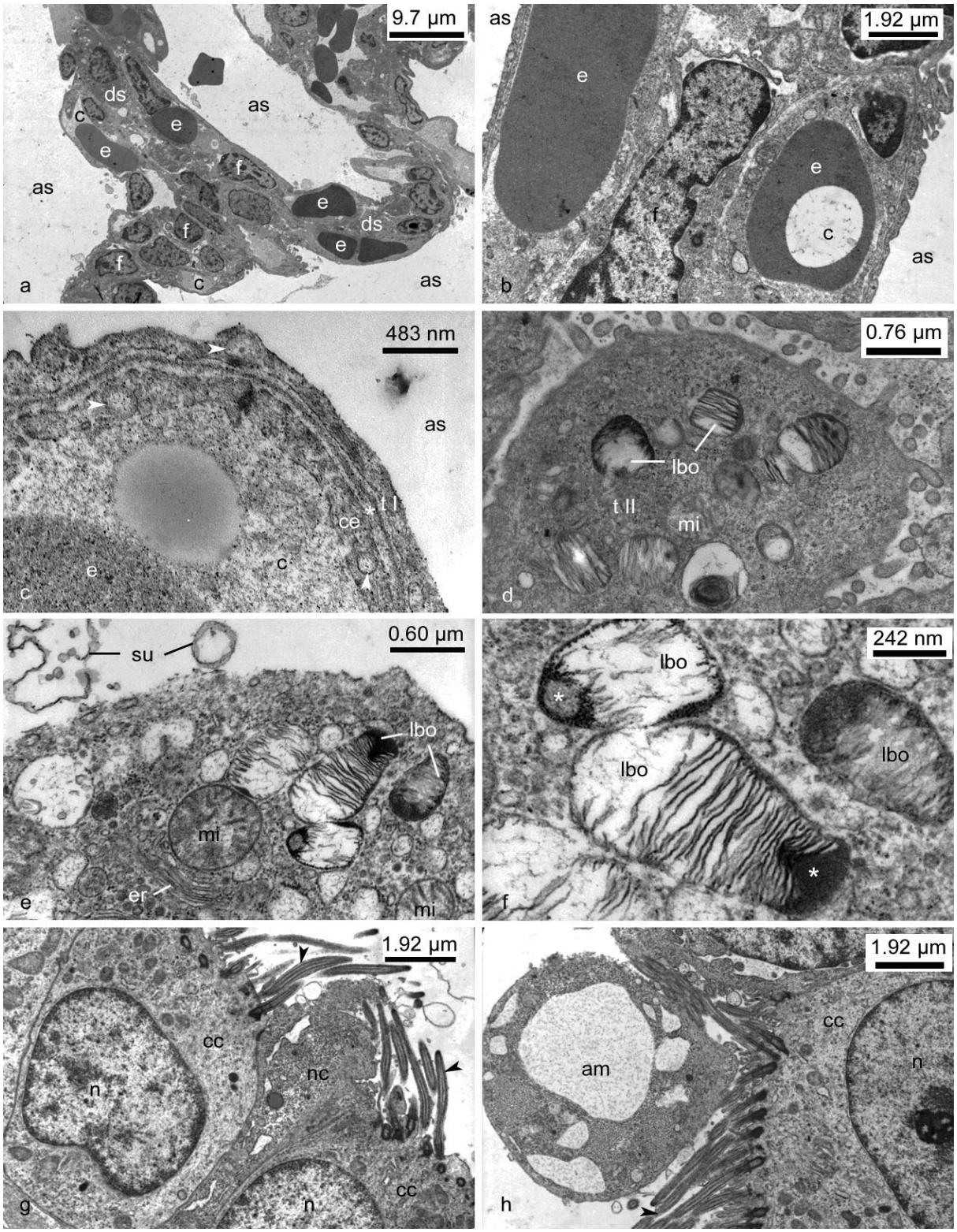

Fig. 64: Transmission electron micrographs of the newborn lung of *Suncus murinus.* The air sacs are separated by septa with a double capillary network with a compact interstitial layer (a, b). The blood-air barrier is trilaminar in structure (capillary endothelium, epithelium and a fused basal lamina (∗) (c, arrowheads show coated vesicles). The cuboidal type II pneumocytes produce phospholipids inside the lamellar bodies (d, f; asterisk indicate the origin of surfactant), which is secreted in form of surfactant (e). The terminal bronchioles are lined with ciliated and non-ciliated cells (g, h; arrowheads show cilia). am, alveolar macrophage; as, air sac; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; er, endoplasmic reticulum; f, fibroblast; lbo, lamellar body; mi, mitochondria; n, nucleus; nc, nonciliated cell; ds, double capillary septum; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

300 µm in diameter in the left lung. They are lined with one-layered columnar ciliated epithelium. Beneath, a thick layer of four to five smooth muscle cells is lying. The thick muscular layer causes contractions, what leads to longitudinal folds in the epithelium. In the wall of the right main bronchus no cartilage is present. The wall of the left main bronchus comprises cartilage only extrapulmonar up to the first dichotomy. The lobar and segmental bronchi measure 200-250 µm and 200 µm in diameter respectively. They are lined with simple cuboidal ciliated epithelium and their walls comprise a layer of two to three smooth muscle cells. The bronchioles measure 60-100 µm in diameter and are lined with simple cuboidal epithelium. They contain few or none muscle cells in their walls. In the distal portion of the terminal bronchioles ciliated and nonciliated cells alternate (Fig. 64 g). However, the sparse covering and the shortness of the cilia indicate an initial stage of ciliation (Fig. 63 h). The apical region of the ciliated cells is characterised by numerous mitochondria and cilia at the surface, whereas the basal region contains the nucleus (Fig. 64 g, h). At the distal portion the terminal bronchioles flatten and form straight and smooth walled channels, which end up in the small terminal air sacs (Fig. 63 f).

The numerous small terminal air sacs in the lung of the newborn *Suncus murinus* measure 60-80 µm in diameter (Fig. 63 d, g). The air sacs appear to be smooth walled. They are lined mainly with the squamous type I pneumocytes. The cuboidal type II pneumocytes are rare and can be found only occasionally interspersed between the type I pneumocytes. The structure of the type II pneumocytes resembles that of the previously described species. Characteristic for type II pneumocytes are the microvilli at their surface, which protrude into the air sac lumen (Fig. 64 d). The type II pneumocytes contain prominent lamellar bodies, which synthesise phospholipids in form of the lamellar surfactant (Fig. 64 d, e, f). In the ultrastructure of the lamellar bodies it becomes obvious that there is a nucleus or a centre at one side of the lamellar bodies, which is the starting point for the synthesis of surfactant (Fig. 64 f). After the production and storage of surfactant inside the lamellar bodies, the surfactant is secreted from the type II pneumocytes into the air sac lumen. At this developmental stage only few free surfactant can be found in the air space (Fig. 64 e). The air sacs are separated by double capillary septa, which have a thickness of 10-15 µm (Fig. 64 a). The septa are straight without folding or branching. In the septa of the neonatal lung of *Suncus murinus* a double capillary network is present. The septa contain a central interstitial layer of connective tissue and fibroblasts flanked on both sides by capillaries (Fig. 64 b). The erythrocytes inside the capillaries are without a nucleus already at birth. The blood-air barrier has a trilaminar structure. It consists of capillary endothelial cells, epithelial cells of type I pneumocytes and a fused basal lamina of both cell types (Fig. 64 c). The diffusion barrier has a thickness of 400-450 nm. Some type I pneumocytes and endothelial cells contain coated vesicles of various size. These vesicles may be involved in the removal of small air borne particles.

The lung of the four days old *Suncus murinus*

In the lung of the four days old *Suncus murinus*, the air sacs seem to be slightly more irregular in shape (Fig. 65 a). Short septal ridges appear on the surface of the air sac septa and form the first alveoli (Fig. 66 a). The light microscopic and electron microscopic findings of the lung structure of a four days old *Suncus murinus* are presented in the figures 65 and 66.

The bronchial tree resembles that of the newborn lung and extends far into the periphery of the lung (Fig. 65 a, b). In the most distal portion of the airways, the terminal bronchioles pass into smooth-walled channels which open directly into the terminal air sacs (Fig. 65 c, e). Transmission electron micrographs reveal the ultrastructure of the terminal bronchioles. The cuboidal epithelium, lining the bronchiolar wall, consists of ciliated and non ciliated cells, which alternate (Fig. 66 g, h). The ciliated cells are characterised by a dense covering with cilia in the apical area and a small nucleus in the basal part of the cell. In contrast, the nonciliated or Clara cells contain a large nucleus, which takes nearly the entire cell, and a few mitochondria in the apical part of the cell (Fig. 66 h). The air sacs of the four days old *Suncus murinus* lung have with a diameter of 60-80 µm the same size as in the newborn lung (Fig. 65 c). However, they are more irregular in shape with short septal crests protruding into the air space (Fig. 66 a). Small buds are sprouting from the air sacs into the surrounding parenchyma and form the first alveoli (Fig. 65 d, f). The new formed alveoli measure 40-50 µm in diameter. They are separated by septal outgrowths, which form single capillary septa (Fig. 66 a). At the age of four days, septa with a double capillary network are still dominating in the lung. In contrast, the new formed septa consist of only one centrally located capillary bed abutting on both sides of the adjacent air space (Fig. 66 b). Compared to the double capillary septa, the single capillary septa are thinner. They measure 7-10 µm in width.

The trilaminar structure of the blood-air barrier resembles that of the diffusion barrier in the newborn lung (Fig. 66 c). The thickness of the blood-air barrier is in attenuated areas 350 nm and in less attenuated areas 550 nm. Numerous coated vesicles are present in the endothelial and epithelial cells of the blood-air barrier. The coated vesicles in the type I pneumocytes are involved in the removal of small air borne particles. For the removal of larger particles and surplus surfactant some alveolar macrophages are moving free in the air space (Fig. 66 f). They possess pseudopodia, which are involved in the movement and phagocytosis of the cell. The large number of lysosomes inside the alveolar macrophages reflects the phagocytotic capacity of these cells. The type II pneumocytes contain numerous lamellar bodies, which synthesise, store and secrete surfactant (Fig. 66 d, e). At the age of four days a lot of surfactant can be found in the air space. That refers to an increased secretion of surfactant at the time of the beginning alveolarisation.

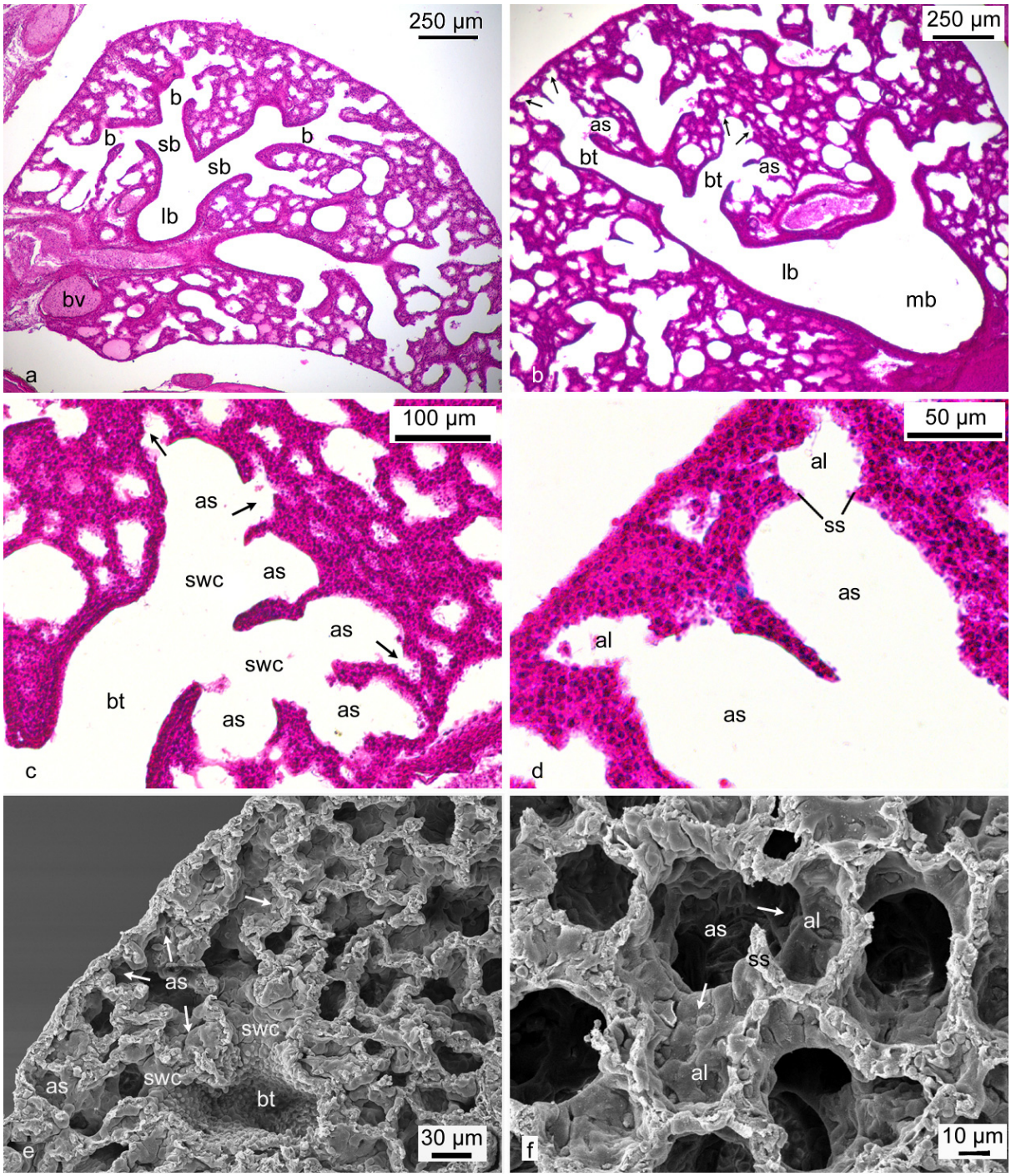

Fig. 65: Light micrographs (a-d) and scanning electron micrographs (e, f) of the lung of a four days old *Suncus murinus*. The lung parenchyma of the four days old lung becomes more and more subdivided and the bronchial tree is widely ramified (a, b). At the age of four days the formation of alveoli starts (c, d, e, f; alveoli are indicated by arrows). The alveoli are small buds sprouting from the air sacs. They are separated from each other by single capillary septa, which arise from the double capillary septa (d, f). al, alveole; as, air sac; b, bronchiole; bt, terminal bronchiole; bv, blood vessel; lb, lobar bronchus; mb, main bronchus; sb, segmental bronchus; ss, single capillary septum; swc, smooth-walled channel. HE staining. Magnification is indicated by the scale bar.

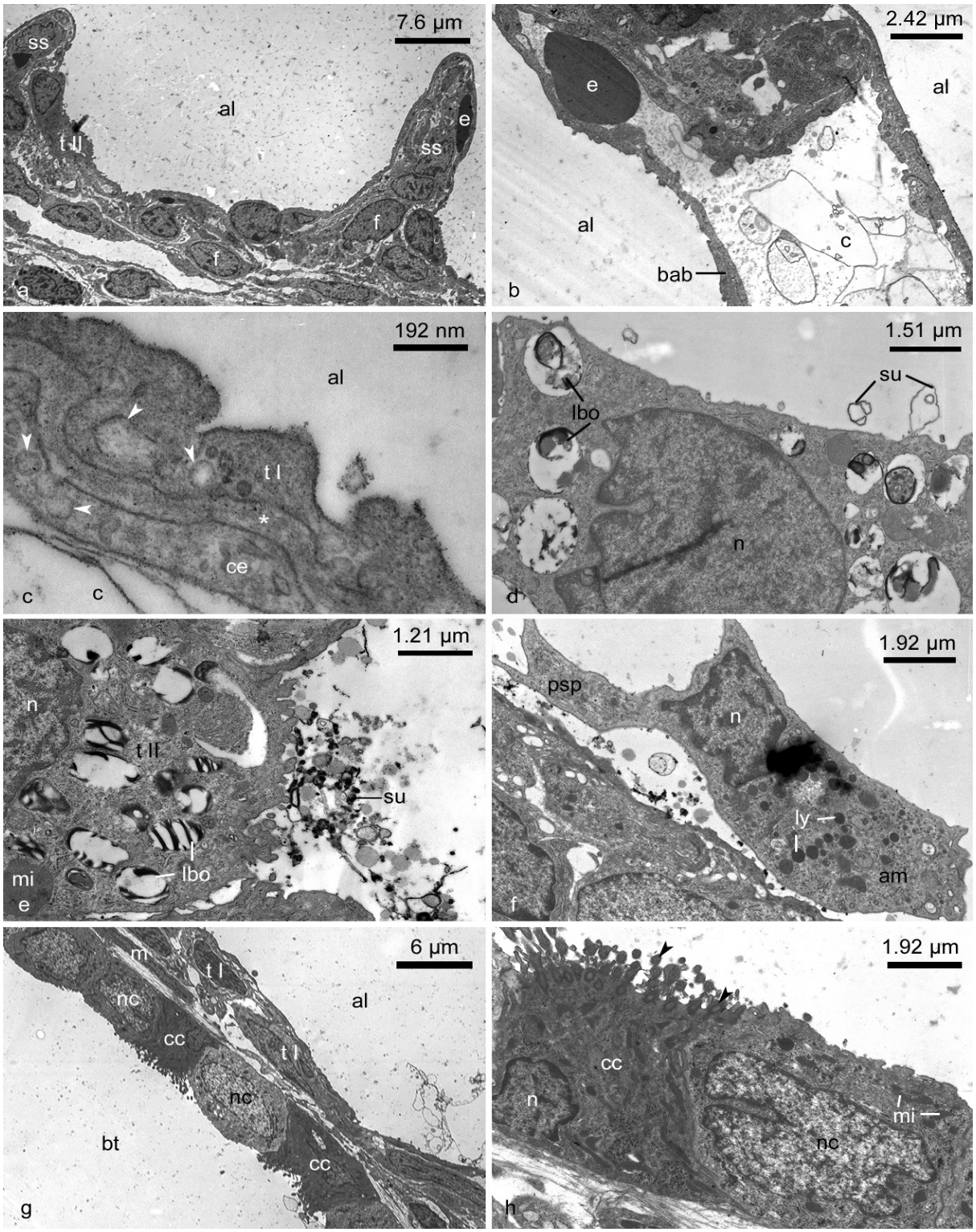

Fig. 66: Transmission electron micrographs of the lung of the four days old *Suncus murinus.* Alveoli are formed by septal outgrowths (a). The new formed septa have a single capillary network (b). The blood-air barrier is similar to that in the newborn lung (c; asterisk indicates the basal lamina; coated vesicles are indicated by arrowheads). The type II pneumocytes contain many lamellar bodies and a lot of secreted surfactant can be found in the air space (d, e). Some macrophages are present in the alveolar lumen (f). In the distal portion of the terminal bronchioles ciliated and nonciliated cells aternate (g, h; arrowheads indicate cilia). al, alveole; am, alveolar macrophage; bab, blood-air barrier; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; ly, lysosome; m, muscle cell; mi, mitochondria; n, nucleus; nc, nonciliated cell; psp, pseudopodia; su, surfactant; ss, single capillary septum; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

The lung of the seven days old *Suncus murinus*

In the lung of the seven days old *Suncus murinus* the formation of alveoli proceeds and the lung parenchyma becomes more and more subdivided (Fig. 68 a, d). At this developmental stage several transformations of the terminal airways can be observed.

A schematic representation of the bronchial tree of *Suncus murinus* at the age of seven days shows figure 67. The light microscopic and electron microscopic findings of the lung of a seven days old *Suncus murinus* are presented in the figures 68 and 69. At the age of seven days the right lung measures 9.3 mm in length, whereas the left lung is with 6.3 mm definitely smaller. Compared to the neonate, the lung size has nearly doubled. Relating to the whole body length of 62 mm, the right lung takes still a seventh part of the whole body length.

The bronchial tree of the seven days old lung resembles that of the neonatal lung (Fig. 67 b). The branching pattern of the lobar bronchi is equivalent to the newborn lung. The schematic representation of the main bronchus of the right lung with middle lobe and accessory lobe bronchi branching off reveals a well differentiated lung structure (Fig. 67 a).The bronchial tubes increase in length and branch into several bronchioles which end in numerous small

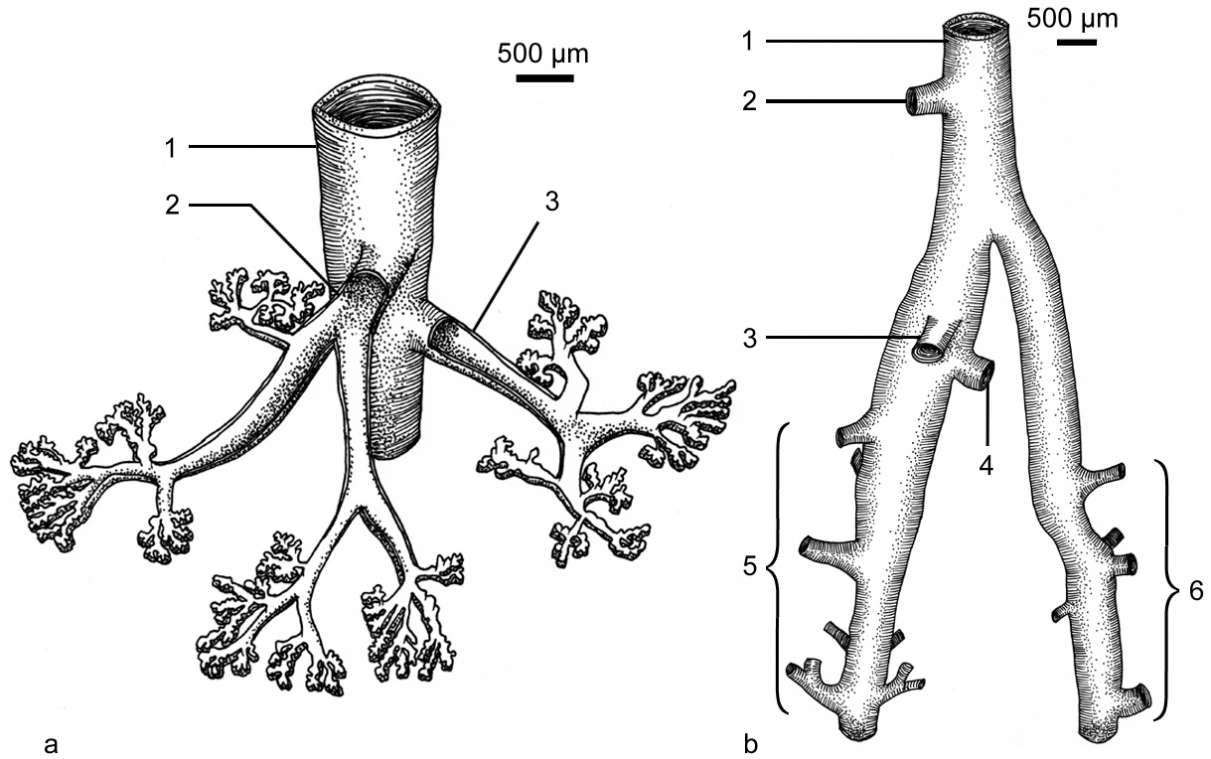

Fig. 67: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the lung of a seven days old *Suncus murinus*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 - inferior lobe bronchi. Magnification 25 x.

alveoli. The structure of the bronchiolar walls is similar to that in the neonatal lung. However, the conducting airways increase in diameter. The right main bronchus measures 700 µm in

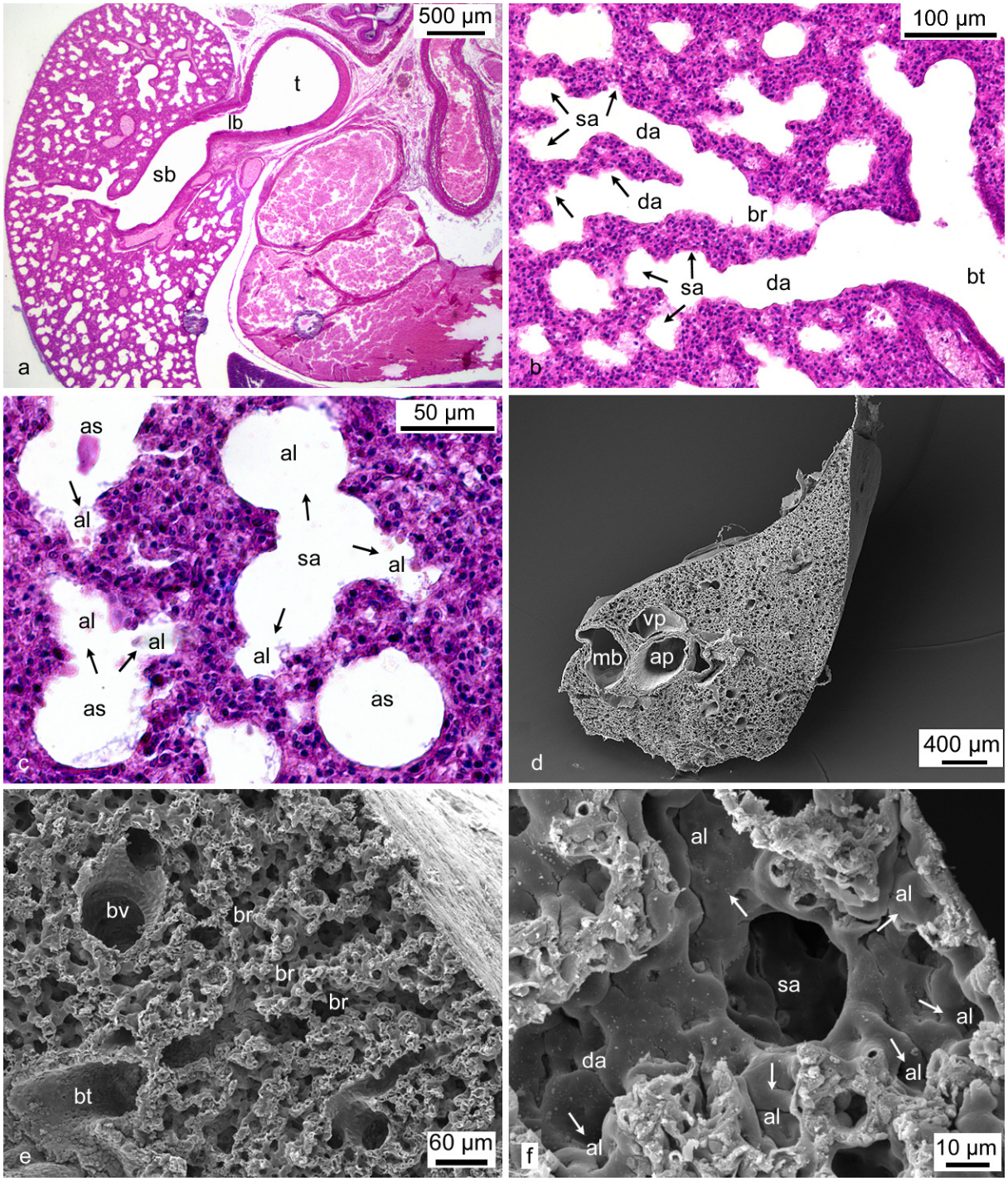

Fig. 68: Light micrographs (a-c) and scanning electron micrographs (d-f) of the lung of a seven days old *Suncus murinus*. The formation of alveoli proceeds and the lung parenchyma becomes more and more subdivided (a, d). New alveoli are also formed in the distal parts of the bronchiolar walls. Respiratory bronchioles with flattened epithelium and shallow depressions in their walls are recognisable (b, e). The previous smooth walled channels and air sacs transform successive into alveolar ducts and alveolar sacs. al, alveole; ap, pulmonary artery; as, air sac; b, bronchiole; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; da, alveolar duct; lb, lobar bronchus; mb, main bronchus; sa, alveolar sac; sb, segmental bronchus; t, trachea; vp, pulmonary vein. HE staining. Magnification is indicated by the scale bar.

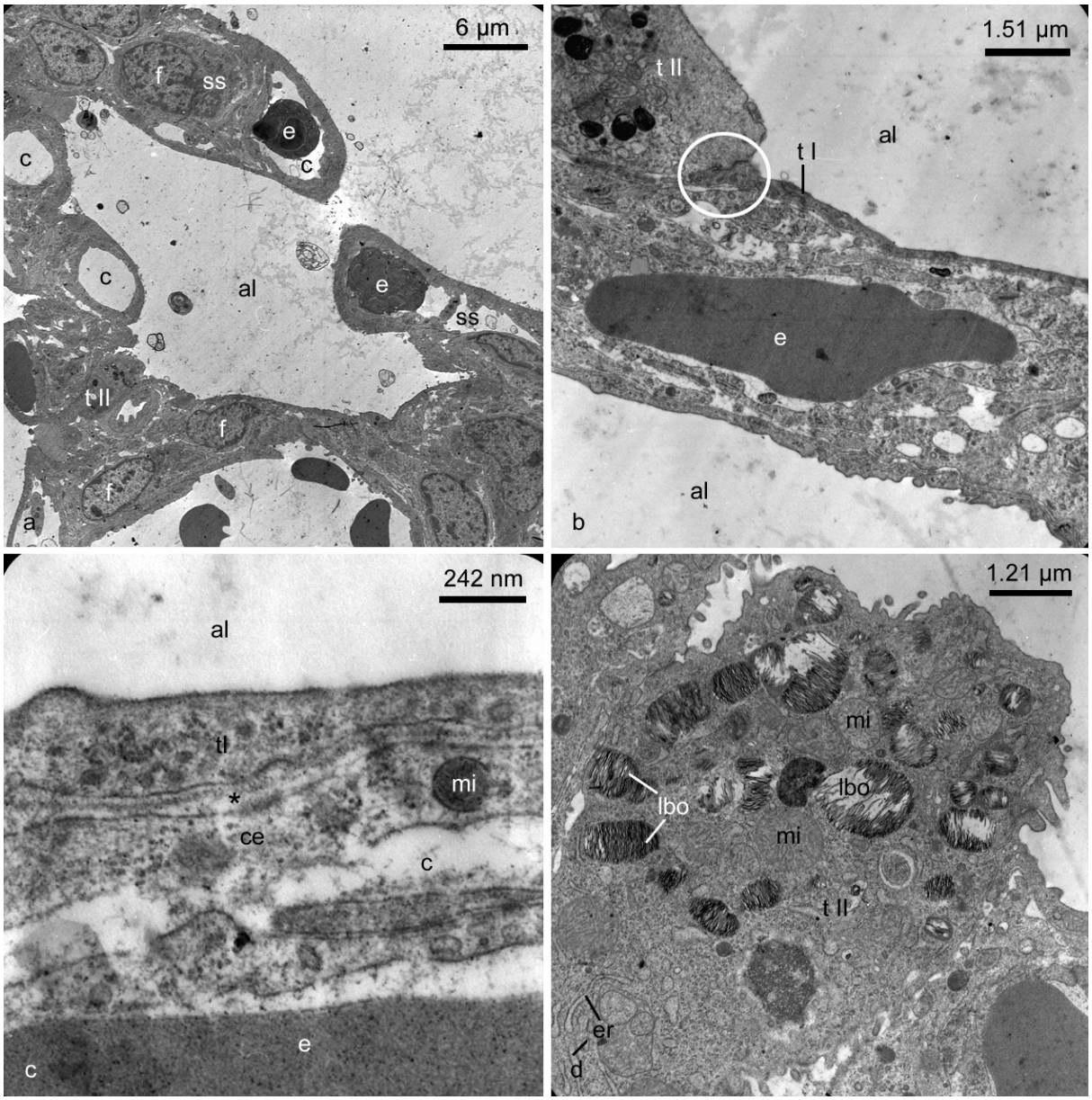

Fig. 69: Transmission electron micrographs of the lung of a seven days old *Suncus murinus.* Alveoli are formed by septal outgrowths (a). Septa with a single capillary network, become more frequent (b). The alveoli are lined with squamous type I pneumocytes and cuboidal type II pneumocytes. The both cell types are attached to each other by tight junctions (b, circle). The blood-air barrier resembles that of the earlier developmental stages (c, asterisk indicates the basal lamina). The type II pneumocytes are characterised by many lamellar bodies, mitochondria and endoplasmic reticulum (d). al, alveole; c, capillary; ce, capillary endothel; e, erythrocyte; er, endoplasmic reticulum; f, fibroblast; lbo, lamellar body; mi, mitochondria; ss, single capillary septum; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

diameter in the proximal part and 500 µm in diameter in the more distal part. The left main bronchus is with 500 µm in diameter comparatively smaller. The lobar bronchi vary in diameter from 250 to 450 µm.

The most remarkable changes occur in the terminal airways. The terminal bronchioles measure 80-100 µm in diameter and are lined with one-layered cuboidal epithelium. In the distal portions of the bronchiolar walls the epithelium flattens and shallow depressions can be

recognised in the walls. From now on these bronchioles are termed respiratory bronchioles (Fig. 68 b, e). They measure 50-80 µm in diameter. Another modification concerns the former smooth-walled channels, which transform into alveolar ducts with alveoli at their sides (Fig. 68 b). They open into the former air sacs, which become now alveolar sacs with alveoli radiating in a concentric way (Fig. 68 c, f).

In the seven days old lung some small terminal air sacs of 70 µm in diameter are still present. But many of the air sacs are already transformed into alveolar sacs and give rise to several alveoli. The alveoli measure 20-30 µm in diameter and are lined with squamous type I pneumocytes and occasionally interspersed type II pneumocytes. The both cell types are attached to each other by junctional complexes (circle, Fig. 69 b). The type II pneumocytes show no structural changes in the intracellular composition. However, the number of the lamellar bodies inside the cells seems to be increased (Fig. 69 d).

The separation of alveoli is resulting from septal outgrowths (Fig. 69 a). These new formed septa contain a single central capillary spanning nearly the entire septum (Fig. 69 b). The thickness of the septum is with 5-10 µm nearly the same as in the four days old lung. The compact interstice contains some fibroblasts. The structure of the blood-air barrier is trilaminar and resembles that of the earlier developmental stages (Fig. 69 c). In the epithelial and endothelial layer some mitochondria and coated vesicles can be recognised. The thickness of the diffusion barrier is approximately 500 nm.

The lung of the 11 and 14 days old *Suncus murinus*

The overall appearance of the lung parenchyma of the 11 and 14 days old *Suncus murinus* is that of a mature lung (Fig. 70 a, e). The histological findings of the lung structure of the 11 and 14 days old *Suncus murinus* are presented in figure 70.

The bronchial tree resembles that of the seven days old *Suncus murinus*. It is consistent in the diameters and the structures of the bronchial and bronchiolar walls. In order to reach also the peripheral areas of the lung, the conducting airways have to increase in length (Fig. 70 b, f).

The formation of alveoli continues and numerous new alveoli are formed in the distal parts of the bronchiolar walls. That leads to an increase in the number of respiratory bronchioles on the one hand, and to the elongation of the respiratory bronchioles on the other hand (Fig. 70 c, g). At the distal end, the respiratory bronchioles pass into alveolar ducts, which open into alveolar sacs (Fig. 70 c).

From the alveolar sacs, several small alveoli radiate in a concentric way (Fig. 70 d, h). The alveolar sacs vary in size. They measure from 25 to 45 µm in diameter. That leads to the

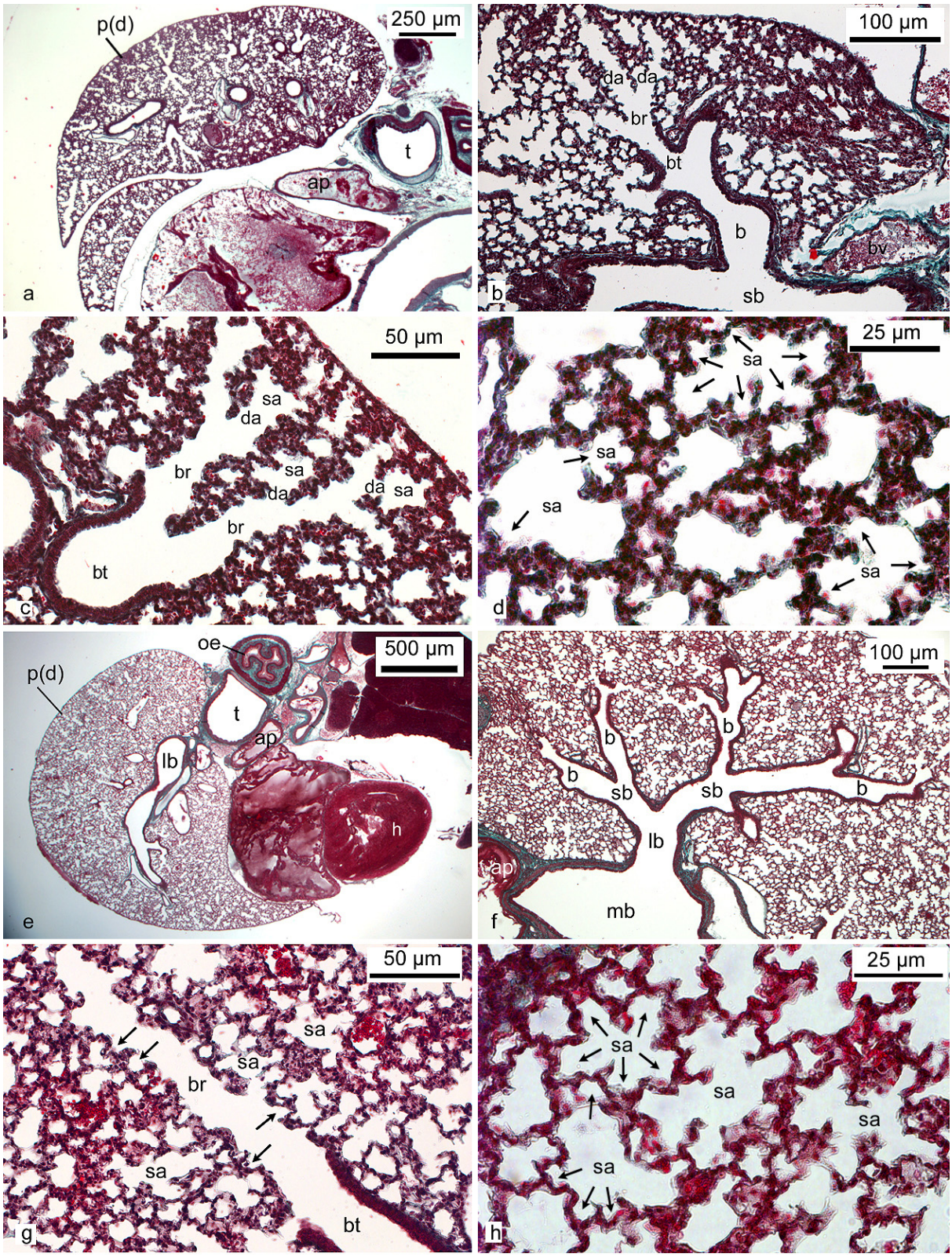

Fig. 70: Light micrographs of the lungs of a 11 (a-d) and a 14 (e-h) days old *Suncus murinus*. The septation of the lung parenchyma has made further progress (a, e). The bronchial tree is well developed and shows many dichotomies (b, f). With the proceeding formation of alveoli respiratory bronchioles and alveolar ducts are more numerous and increase in length (c, g). The alveolar ducts open into alveolar sacs, from which alveoli radiate (d, h; alveoli are indicated by arrows). al, alveole; ap, pulmonary artery; b, bronchiole; bt, terminal bronchiole; br, respiratory bronchiole; da, alveolar duct; h, heart; lb, lobar bronchus; mb, main bronchus; p(d), right lung (pulmo dextra); sa, alveolar sac; sb, segmental bronchus; t, trachea. Trichrome staining. Magnification is indicated by the scale bar.

assumption, that several generations of alveolar sacs are present. Some are transformed from original air sacs and others were derived from alveoli. The new formed alveoli are small and measure only 8-12 µm in diameter.

The lung of the adult *Suncus murinus*

The main changes occuring after day 14 are the expansion and the thinning of the alveolar septa. The lung parenchyma of the adult lung appears to be highly subdivided (Fig. 71 a). The light microscopic and electron microscopic findings of the adult lung of *Suncus murinus* are presented in the figures 71 and 72.

The bronchial tree of the adult lung shows no structural changes. The diameters and structural composition of the bronchial and bronchiolar walls correspond mainly to these in the seven days old lung. However, the lobar bronchi become more muscular. A thick layer of five to seven smooth muscle cells stabilises the bronchial wall (Fig.71 b). The lobar bronchi are lined with one-layered columnar ciliated epithelium. The bronchioles of the adult lung measure 80-100 µm in diameter and are lined with one-layered cuboidal ciliated epithelium. The larger bronchioles contain a circular layer of two to three smooth muscle cells in their walls. The terminal bronchioles have only one layer or no smooth muscle cells in their wall. They measure 60-80 µm in diameter and in the distal portions they pass into respiratory bronchioles, which have alveoli at their sides. The respiratory bronchioles pass into alveolar ducts which open in large alveolar sacs (Fig. 71 c). The postnatal development of the alveolar region involved particularly the organisation, growth, and surface enlargement of the gas-exchanging structures. In the adult lung the supseptation of the respiratory airspaces has progressed considerably. Compared to the 14 days old lung, the alveolar sacs have increased in size. They measure now 40-80 µm in diameter and are more irregular in shape. From the alveolar sacs numerous alveoli radiate (Fig. 71 d, e). The alveoli of the adult lung vary in size. There are small alveoli with a diameter of 10 µm, but also larger alveoli up to 40 µm in diameter. The septa separating the alveoli become thinner and measure now 5-7 µm in width. The slender septa of the adult lung contain a single capillary system, which runs central or alternately on the left or on the right side of a connective tissue sheet (Fig. 71 f, 72 a, b).

The blood-air barrier of the adult lung has a thickness of approximately 500 nm and retains the trilaminar structure of the earlier developmental stages (Fig. 72 c). However, in comparison to earlier developmental stages the basal lamina of the adult lung is relatively thick. Endothelial and epithelial cells contain numerous coated vesicles.

The type II pneumocytes of the adult lung show the typical cell structure. But, compared to earlier developmental stages, the type II pneumocytes contain a higher number of lamellar

bodies (Fig. 72 d).

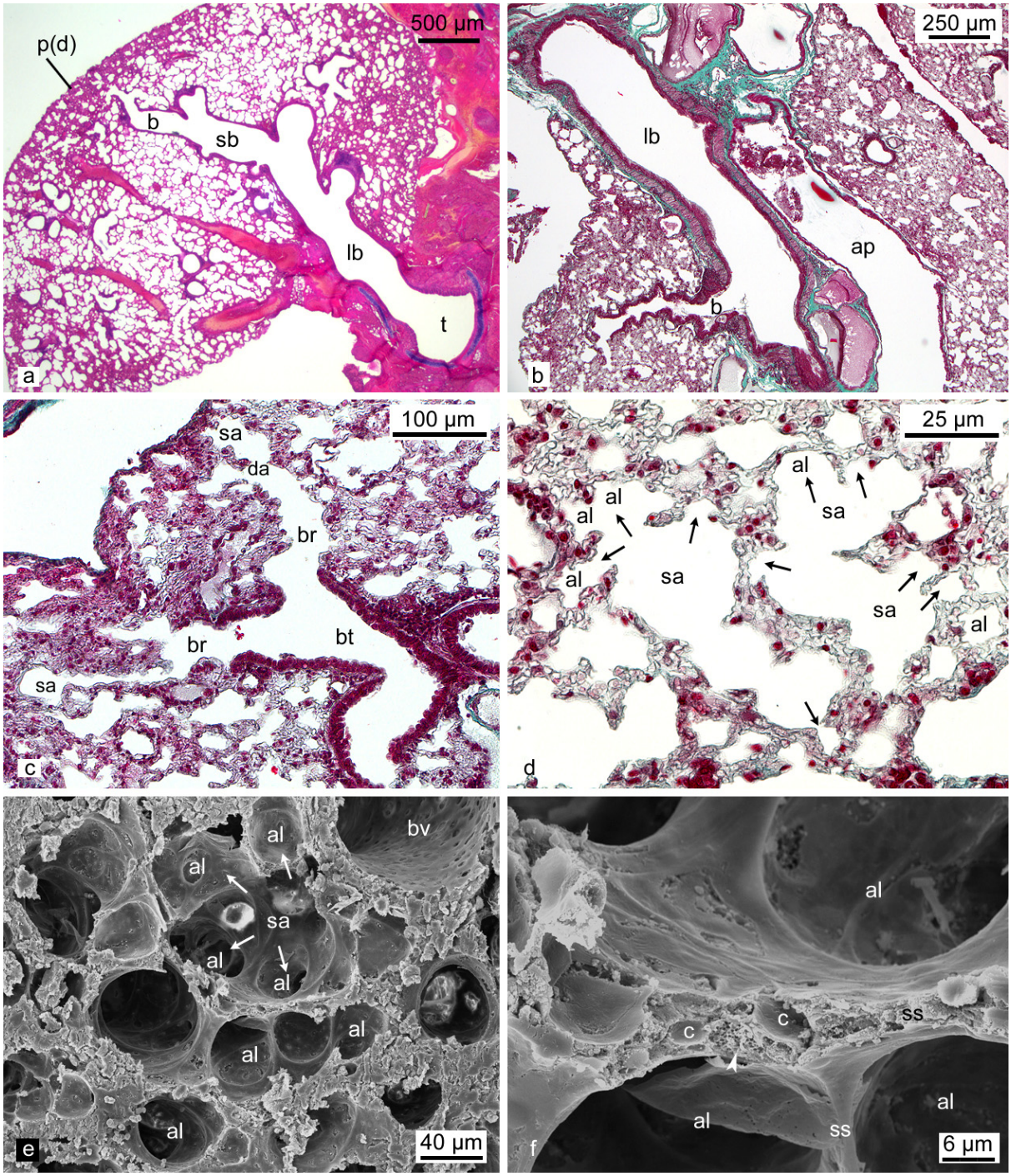

Fig. 71: Light micrographs (a-d) and scanning electron micrographs (e, f) of the lung of a adult *Suncus murinus*. The mature lung is highly subdivided (a). The numerous alveoli radiate from alveolar sacs (d, e) and are separated by thin single capillary septa (f, arrowhead indicates a type II pneumocyte). The alveoli vary in size from 10 to 40 µm in diameter. That reflects different developmental stages of the alveoli. Compared to the 14 days old lung a general size increase of the alveoli took place. The bronchial tree resembles that of the earlier stages, but the large bronchi are more muscular (b). The terminal airways consist of terminal bronchioles, respiratory bronchioles and alveolar ducts which end in alveolar sacs (c). al, alveole; ap, pulmonary artery; as, air sac; b, bronchiole; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; c, capillary; da, alveolar duct; lb, lobar bronchus; p(d), right lung (pulmo dextra); sa, alveolar sac; sb, segmental bronchus; ss, single capillary septum; t, trachea; vp, pulmonary vein. a: HE; b-d Trichrome staining. Magnification is indicated by the scale bar.

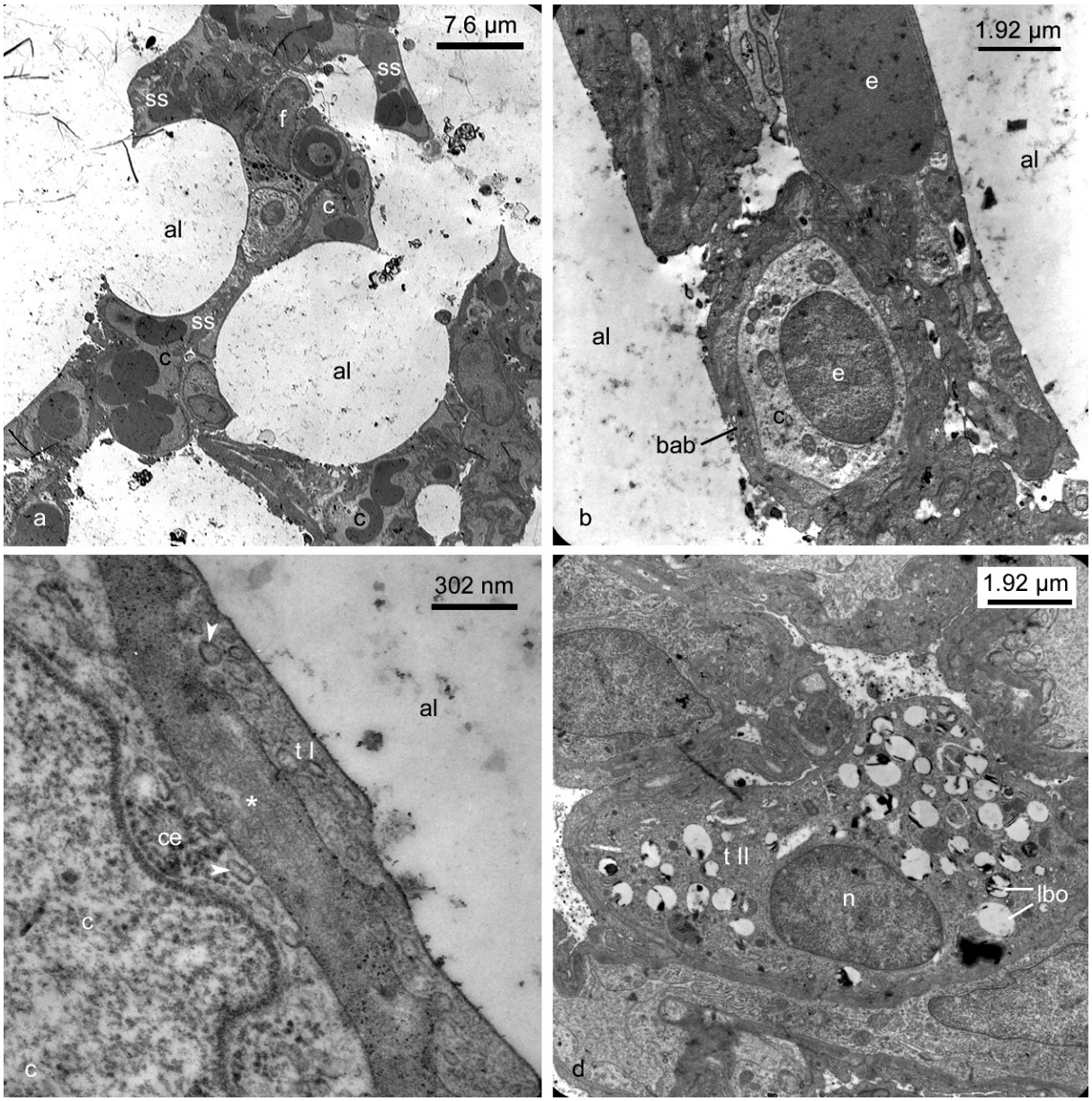

Fig. 72: Transmission electron micrographs of the lung of a adult *Suncus murinus.* Alveoli sprout into the surrounding parenchyma. They are separated by septa with a single capillary bed (a, b). The structure and thickness of the blood-air barrier is similar to that of the seven days old lung (c, asterisk indicates the basal lamina, arrowheads show coated vesicles). The type II pneumocytes contain a comparatively high number of lamellar bodies (d). al, alveole; bab, blood-air barrier; c, capillary; ce, capillary endothel; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; ss, single capillary septum; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

3.2.2.3 Tupaia belangeri

The newborn Tupaia belangeri has closed eyes and lacks fur. It stays in a nest and is left by the female for long periods of 48 hours. Although young Tupaia belangeri are nidicolous for the first month of life, they have to be capable of thermoregulation. The structural change during the early postnatal lung development of Tupaia belangeri is shown in figure 73.

At birth the lung of *Tupaia belangeri* is already at the alveolar stage of lung development

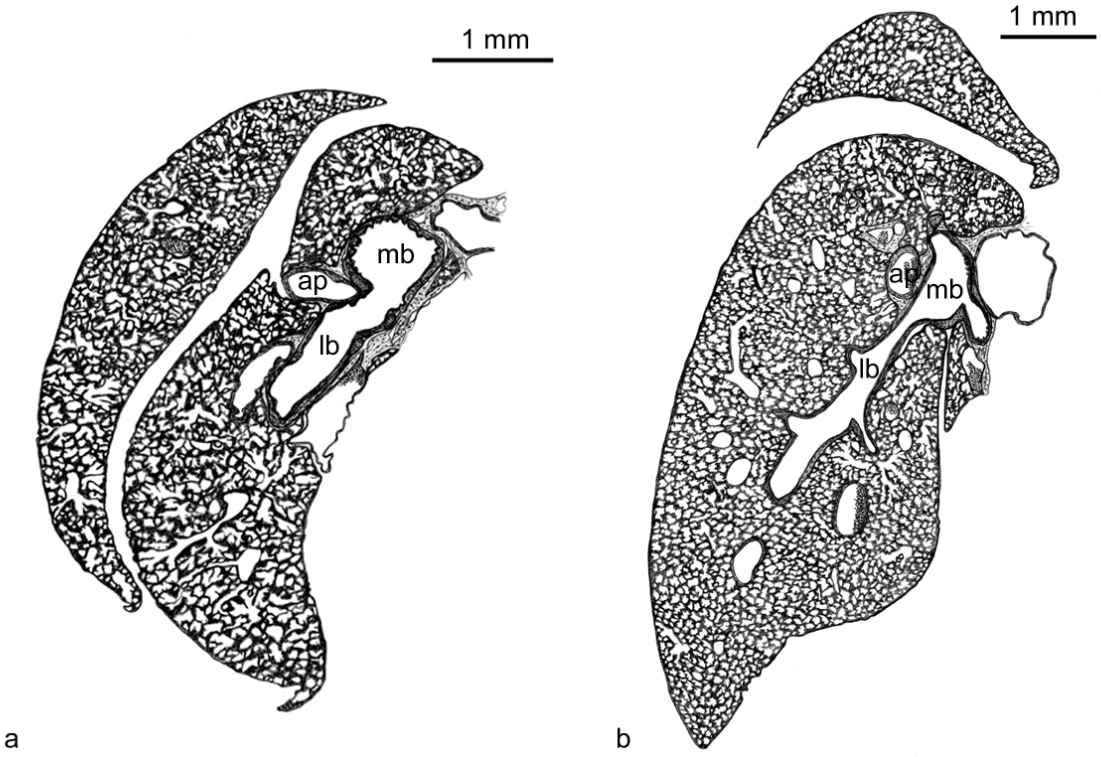

Fig. 73: Lung structure of *Tupaia belangeri* during the early postnatal development. Original drawings from histlogical sections of the right lung of a neonate (a) and a seven days old (b) *Tupaia belangeri.* ap, pulmonary artery; lb, lobar bronchus; mb, main bronchus. Magnification 25 x.

(Fig. 73 a). However, in addition to the numerous alveoli some small terminal air sacs are still present in the newborn lung. At birth the conducting airways are well developed and the ramified bronchial tree extends far to the periphery of the lung.

Due to the advanced development of the lung at birth, the postnatal maturation of the lung comprises simply the multiplication of the alveoli and the proceeding formation of respiratory bronchioles, alveolar ducts and alveolar sacs. In the lung of the seven days old *Tupaia belangeri* the septation has made further progress (Fig. 73 b). The following detailed description of the lung development of *Tupaia belangeri* is subdivided in the postnatal developmental stages examined.

The lung of the neonatal *Tupaia belangeri*

The lung of the newborn *Tupaia belangeri* is already at the alveolar stage of lung development. The lung parenchyma is highly subdivided (Fig. 75 a, e). A schematic reconstruction of the neonatal bronchial tree is shown in figure 74. The light microscopic and electron microscopic findings of the lung structure of the neonatal *Tupaia belangeri* are presented in the figures 75 and 76.

The right and the left lung of the neonatal *Tupaia belangeri* are similar in size. In total length,

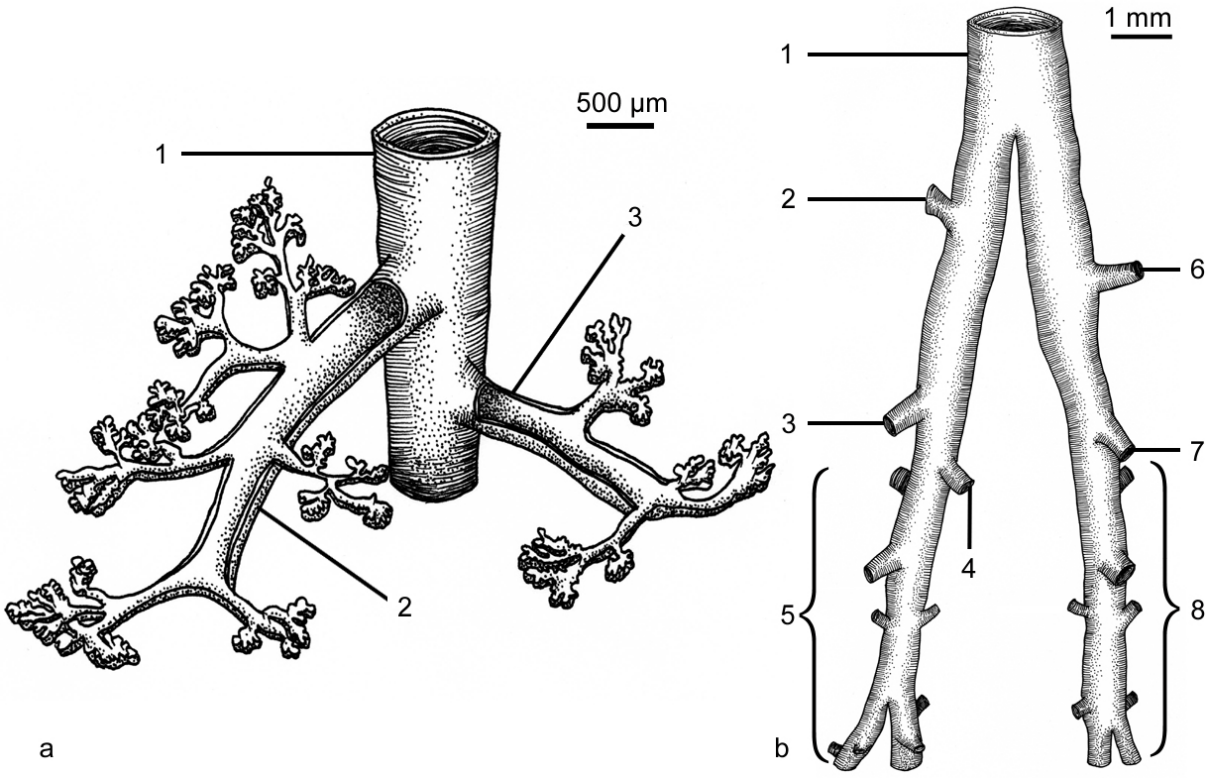

Fig. 74: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe bronchus and accessory lobe bronchus branching off (a) and of the bronchial tree (b) of the lung of the neonatal *Tupaia belangeri*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 - superior lobe bronchus; 7 – middle lobe bronchus; 8 – inferior lobe bronchi. Magnification 25 x.

they measure 10.7 and 10.6 mm respectively. Relating to the CRL of 60 mm, the right lung takes a sixth part of the whole body length.

The right lung of *Tupaia belangeri* consists of completely separated superior, middle, inferior and accessory lobes. The left lung also consists of completely separated superior, middle and inferior lobes. The branching pattern of the lobar bronchi indicates the several areas of supply (Fig. 74 b). In the right lung the superior lobe bronchus is formed by the first dorso-lateral branch from the main bronchus. The middle lobe is supplied by the next branch of the lateral side of the main bronchus. Beneath, on the ventral side of the main bronchus, a bronchus for the supply of the accessory lobe is branching off. The remaining bronchi following in the distal portion of the right main bronchus compose the inferior lobe bronchi. In the left lung, the superior lobe bronchus is similar to that of the right lung, butarises more caudally than that of the right lung. The next bronchus branching off from the lateral side of the main bronchus supplies the middle lobe of the left lung. Several bronchi for the supply of the inferior lobe are branching off in the distal portion of the left main bronchus.

The main bronchi measure 800 µm in diameter in the proximal portions and 600 µm in diameter in the distal portions. They are lined with one-layered columnar ciliated epithelium

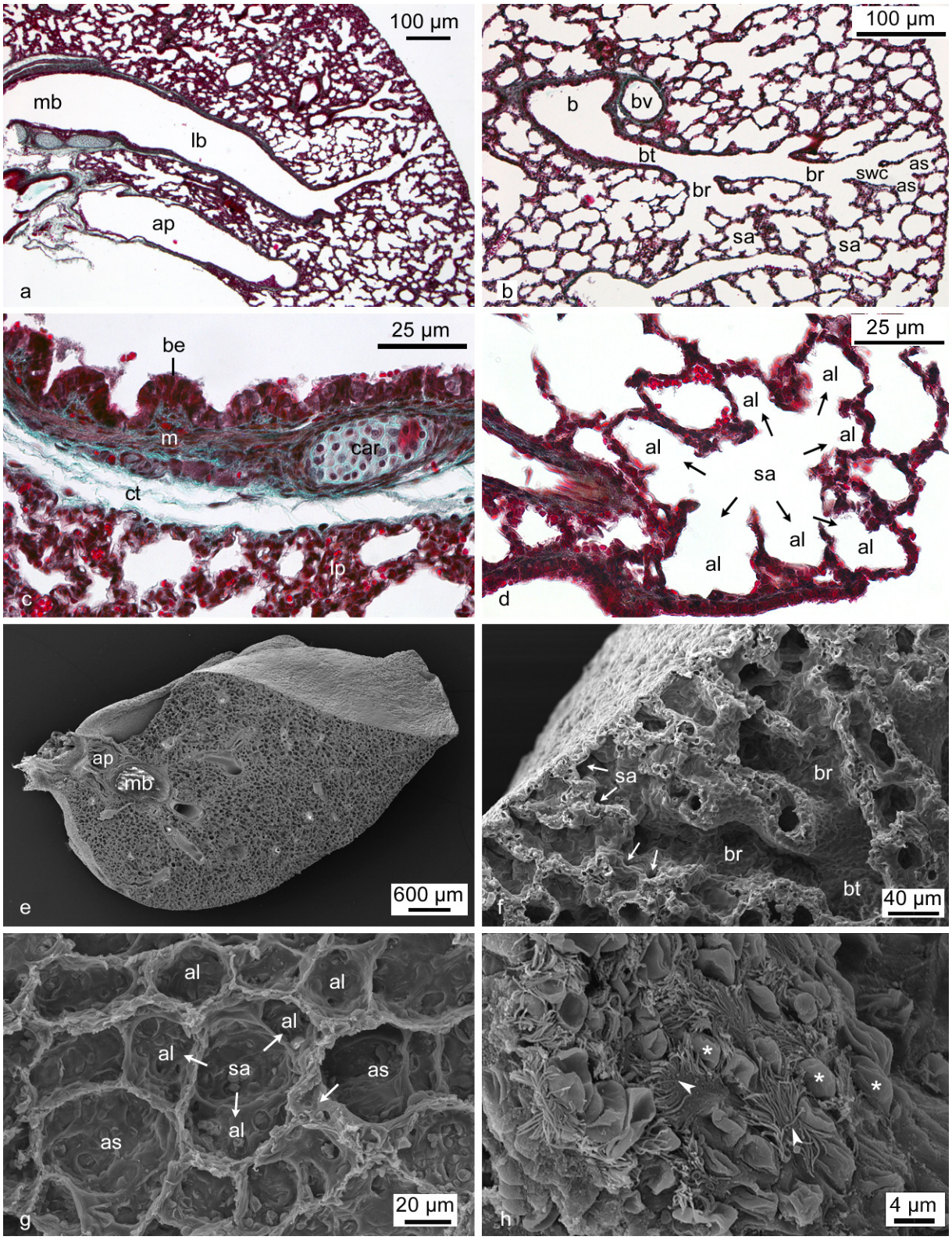

Fig. 75: Light micrographs (a-d) and scanning electron micrographs (e-h) of the newborn lung of *Tupaia belangeri*. The lung is already at the alveolar stage and appears to be subdivided (a, e). Beside numerous small alveoli also air sacs are present (b, d, g). The bronchial tree extends to the periphery of the lung (a, b, f). The formation of respiratory bronchioles seems to has just started (b, f). In the distal portion of the terminal bronchioles ciliated (arrowhead) and nonciliated cells (asterisk) can be found (h). ap, pulmonary artery; al, alveole; as, air sac; b, bronchiole; be, bronchial epithelium; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; car, cartilage; ct, connective tissue; lb, lobar bronchus; lp, lung parenchyma; m, muscle cell; mb, main bronchus; sa, alveolar sac; swc, smooth-walled channel. Trichrome staining. Magnification is indicated by the scale bar.

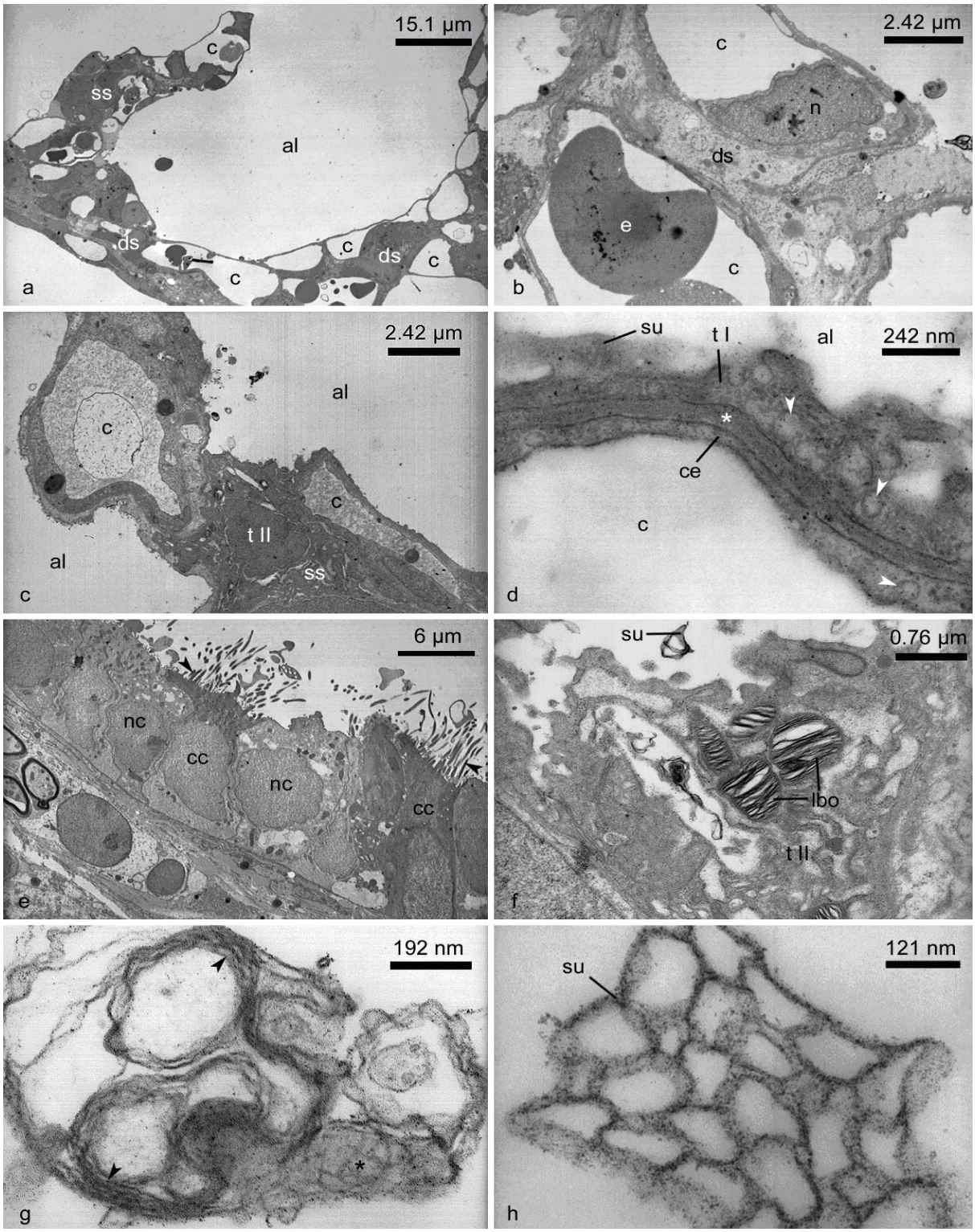

Fig. 76: Transmission electron micrographs of the newborn lung of *Tupaia belangeri.* In the neonatal lung alveoli are already present. They are separated by septal outgrowths, which contain a single capillary network (a, c). But there are still terminal air sacs, which are separated by septa with a double capillary network (b). The trilaminar blood-air barrier consists of capillary endothelium, alveolar epithelium and a fused basal lamina (∗) (d, arrowheads show coated vesicles). The type II pneumocytes synthesise surfactant inside the lamellar bodies (f). Secreted surfactant transforms from lamellae (arrowhead) into a thin film (asterisk) (g) or a special reticular form (h). In the terminal bronchioles ciliated and non-ciliated cells alternate (e; arrowheads show cilia). al, alveole; c, capillary; cc, ciliated cell; ce, capillary endothel; e, erythrocyte; lbo, lamellar body; n, nucleus; nc, nonciliated cell; ds, double capillary septum; ss, single capillary septum; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

(Fig. 75 c). Beneath, a thick layer of three to four smooth muscle cells is lying. The contraction of the muscular layer causes longitudinal folds in the bronchial epithelium. Cartilage supports the walls of the main bronchi. It occurs also intrapulmonar and extends up to the outlets of the first inferior lobe bronchi. Loose connective tissue encloses the cartilage and submucosal glands and separates the main bronchi from the surrounding lung parenchyma. The lobar bronchi are similar in structure, but smaller in size. They measure 200-400 µm in diameter. The walls of the lobar bronchi are lined with one layered cuboidal or columnar ciliated epithelium and comprise a muscular layer of two to three smooth muscle cells. In the proximal parts of the larger lobar bronchi cartilage stabilises the wall. The bronchioles of the newborn lung measure approximately 100 µm in diameter and are lined with one-layered cuboidal epithelium. Their walls comprise only a thin layer of one to two smooth muscle cells. The terminal airways consist mainly of terminal bronchioles and a few respiratory bronchioles (Fig. 75 b). The terminal bronchioles measure 50-70 µm in diameter. Their cuboidal lining epithelium consists of ciliated and nonciliated or Clara cells, which alternate (Fig. 75 h, 76 e). The ciliated cells are characterised by long cilia in their apical region and a large nucleus in the basal part of the cell. The ciliated cells move secretions and trapped airborne particles towards the pharynx. The nonciliated cells bulge into the bronchiolar lumen and have mitochondria and granules in their apical part and a large nucleus in the basal part. Their function is the secretion of material lining the bronchiolar lumen, such as proteins for defense and for breaking up the mucus produced by the upper airways. In the distal parts of terminal bronchioles the epithelium of the bronchiolar walls flatten and form straight and smooth-walled channels, which end up in small terminal air sacs or in the already developed air sacs. In some bronchiolar walls a few shallow depressions can be recognised (Fig. 75 f). These first respiratory bronchioles are rare and not very distinct yet. The respiratory bronchioles measure approximately 40 µm in diameter.

In the newborn lung of *Tupaia belangeri* alveoli are already present. The alveoli are lined with squamous type I pneumocytes and occasional interspersed cuboidal type II pneumocytes. The type II pneumocytes contain some large lamellar bodies (Fig. 76 f). The principal duty of the lamellar bodies is to synthesise, store and secrete surfactant, which mainly consists of phospholipids. The surfactant is secreted by exocytosis from the lamellar bodies. In the air space the lamellae unfold and transform into a thin film, which is the primary surface-active component (Fig. 76 g). In the newborn *Tupaia belangeri* also a special reticular form of surfactant can be observed (Fig. 76 h).

In the newborn lung of *Tupaia belangeri,* beside alveoli, some small air sacs are still present. They measure 30-50 µm in diameter and are separated from each other by double capillary septa. The septa contain a thin central interstitial layer flanked on both sides by capillaries and have a thickness of 10-15 µm (Fig. 76 b). The numerous alveoli are dominating in the

neonatal lung. The alveoli radiate in a concentric way from the air sacs. This leads to a successive transformation from the former air sacs into alveolar sacs (Fig. 75 d, g). The alveoli measure 15-25 µm in diameter. They are formed by sprouting from the air sacs and are separated from each other by septal outgrowths (Fig. 76 a). These septa measure approximately 6 µm in thickness. They consist of a single capillary bed abutting on both sides on the adjacent air space, separated from it only by a thin blood-air barrier (Fig. 76 c). The blood-air barrier in the newborn *Tupaia* lung measures only 200-250 nm in thickness. It is composed of three components, endothelial cells, type I pneumocytes and a fused basal lamina of both cell types (Fig. 76 d). As a fourth component surfactant can be added. The surfactant covers the apical surface of the type I pneumocytes. Endothelial and epithelial cells contain both coated vesicles, which are involved in the transport of proteins or in the removal of small air borne particles.

The lung of the four days old *Tupaia belangeri*

In the lung of the four days old *Tupaia belangeri* the alveolarisation is proceeding. The number of alveoli increases and the terminal portions of the conducting airways transform successively into respiratory airways. The histological and ultrastructural findings of the lung of a four days old *Tupaia belangeri* are shown in figure 77.

The bronchial tree of the four days old lung resembles that of the newborn lung and the diameters and compositions of the bronchial and bronchiolar walls are similar. New alveoli are formed in the distal parts of the bronchiolar walls. As a result respiratory bronchioles with flattened epithelium and alveoli in their walls become more distinct (Fig. 77 a, c). In addition the former smooth-walled channels, which led to the air sacs and alveolar sacs, become alveolarised too. They are termed alveolar ducts by now.

The alveolar sacs are the dominating structures of the terminal air spaces. From the centre of the alveolar sacs numerous alveoli radiate in a concentric way (Fig. 77 b). The alveoli measure still 15-25 µm in diameter and are separated by septa, which contain a single capillary network (Fig. 77 d, f). The new septa are formed mainly by outgrowths from double capillary or older single capillary septa. But there is also some evidence for a transition from the double capillary septa into septa with a single capillary bed. This transformation is achieved by the interconnection and fusion of the opposite capillaries (Fig. 77 e).

In the type II pneumocytes of the four days old lung the synthesis and secretion of surfactant is continued. As the surfactant is synthesised, it is sequestered within granules in a lipid bilayer lamellar form. These lamellar bodies release their contents by exocytosis (Fig. 77 g). Free in the air space, the surfactant relaxes and apears in different structures, such as a reticular form (Fig. 77 h).

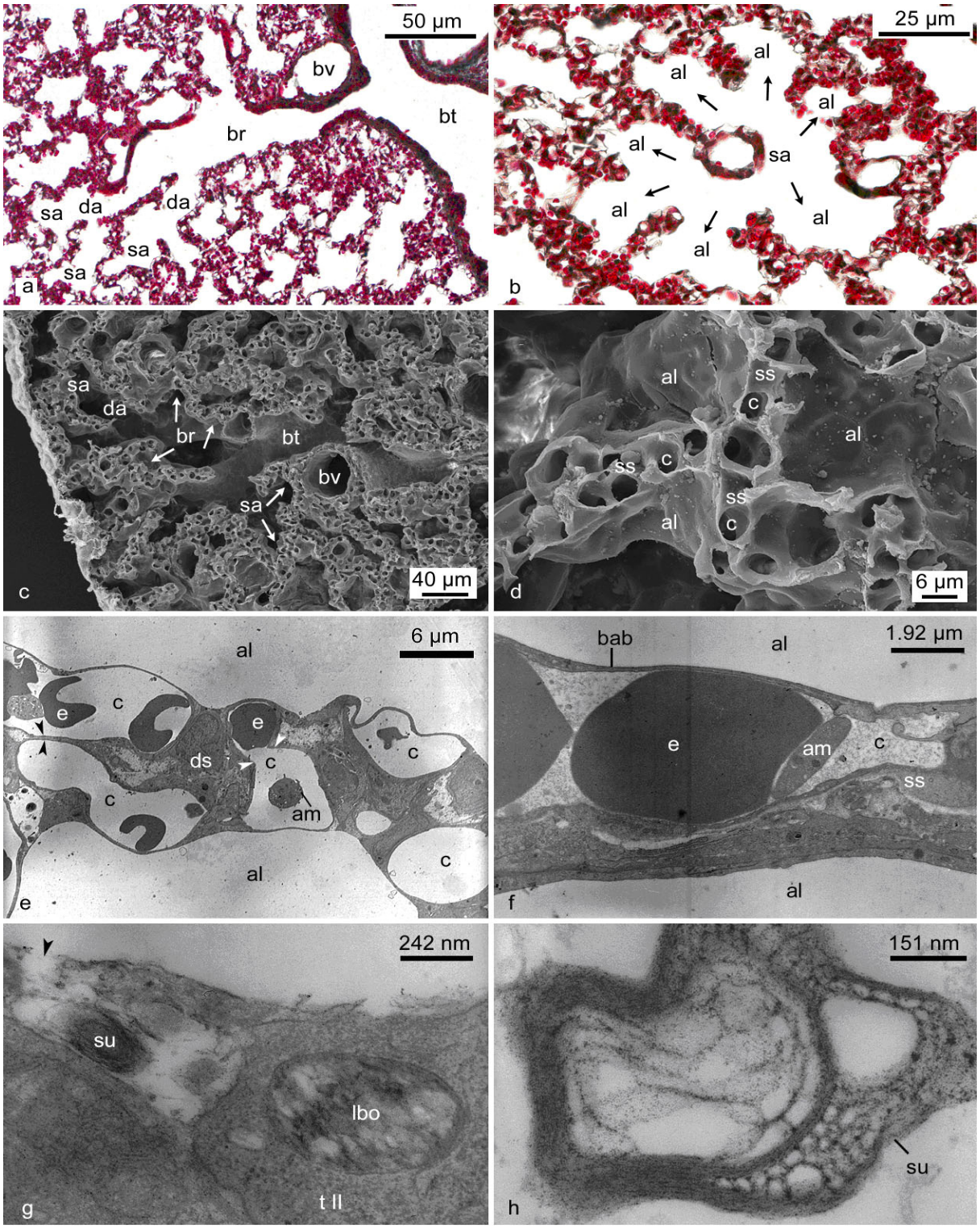

Fig. 77: Light micrographs (a, b) and electron micrographs (c-h) of the lung of a four days old *Tupaia belangeri*. The terminal portions of the airways develop and respiratory bronchioles and alveolar ducts are recognisable (a, c). The number of alveoli increases and alveolar sacs are common (b). The alveoli are separated by septa with a single capillary bed (d, f). The few double capillary septa transform into single capillary septa by fusion of the opposite capillaries (e; arrowheads indicate junctures). Surfactant is secreted by the type II pneumocytes (g; exocytosis is indicated by arrowhead) and the free surfactant shows a reticular form (h). al, alveole; am, alveolar macrophage; bab, blood-air barrier; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; c, capillary; da, alveolar duct; e, erythrocyte; lbo, lamellar body; ds, double capillary septum; sa, alveolar sac; ss, single capillary septum; su, surfactant; t II, type II pneumocyte. Trichrome staining. Magnification is indicated by the scale bar.

The lung of the seven days old *Tupaia belangeri*

At this stage of pulmonary development, the subseptation of the respiratory airspaces has progressed considerably (Fig. 78 a, e). Respiratory bronchioles, alveolar ducts and alveolar sacs are well developed and numerous. The light microscopic and electron microscopic findings of the seven days old lung of *Tupaia belangeri* are presented in the figures 78 and 79.

The main, lobar and segmental bronchi and the bronchioles have nearly the same diameters and compositions of the walls as already described for the neonatal lung. There are no structural changes apparent. However, the cartilage extends further down in the lobar bronchi (Fig. 78 a). In the distal portions of the terminal bronchioles a dense covering with cilia is present (Fig. 78 g). The ultrastructural investigation reveals alternating ciliated and nonciliated cells (Fig. 79 h). The ciliated cells possess long cilia on their apical surface and a nucleus in the basal part of the cell. In contrast, the nonciliated cells have smooth surfaces, which bulge into the bronchiolar lumen. They contain a large nucleus, which occupies nearly the entire cell. The alveolarisation of the distal portions of the terminal airways has progressed. The alveolar outpocketings in the walls of respiratory bronchioles and alveolar ducts become deeper and clearly recognisable (Fig. 78 b, c, f). Due to the continued formation of alveoli in the distal airways, also the number and the length of respiratory bronchioles increase.

The centripetal partitioning of the air spaces proceeds and the number of alveolar sacs increases. From the centre of the alveolar sacs numerous alveoli radiate (Fig. 78 d, h). The alveoli measure 10-30 µm in diameter. This variation in size indicates a further subseptation of older alveoli. An outgrowing arched crest divides the lumen of an alveole and separates the two new formed alveoli from each other (Fig. 79 a). The septa separating the alveoli are septa, with a single capillary spanning the entire septum (Fig. 79 b). As alveolarisation proceeds, the septa gradually become thinner; they measure 4-6 µm in thickness. The centrally located capillary is abutting on both sides on the adjacent air space. The structure and thickness of the blood-air barrier is similar to that described in the neonatal lung (Fig. 79 c). The type II pneumocytes are still active in synthesises and secretion of surfactant. However, some of the type II pneumocytes contain many lysosomes (Fig. 79 d). Type II pneumocytes are the major cells responsible for the turnover of surfactant. The lysosomes inside the type II pneumocytes may be a part of an endocytotic recycling pathway of the cell. The already secreted surfactant transforms in the alveolar lumen from lamellae into a thin film or a reticular form (Fig. 79 e, f). The surplus of surfactant in the alveolar lumen is removed by phagocytosis of alveolar macrophages, which occur free in the air space (Fig. 79 g).

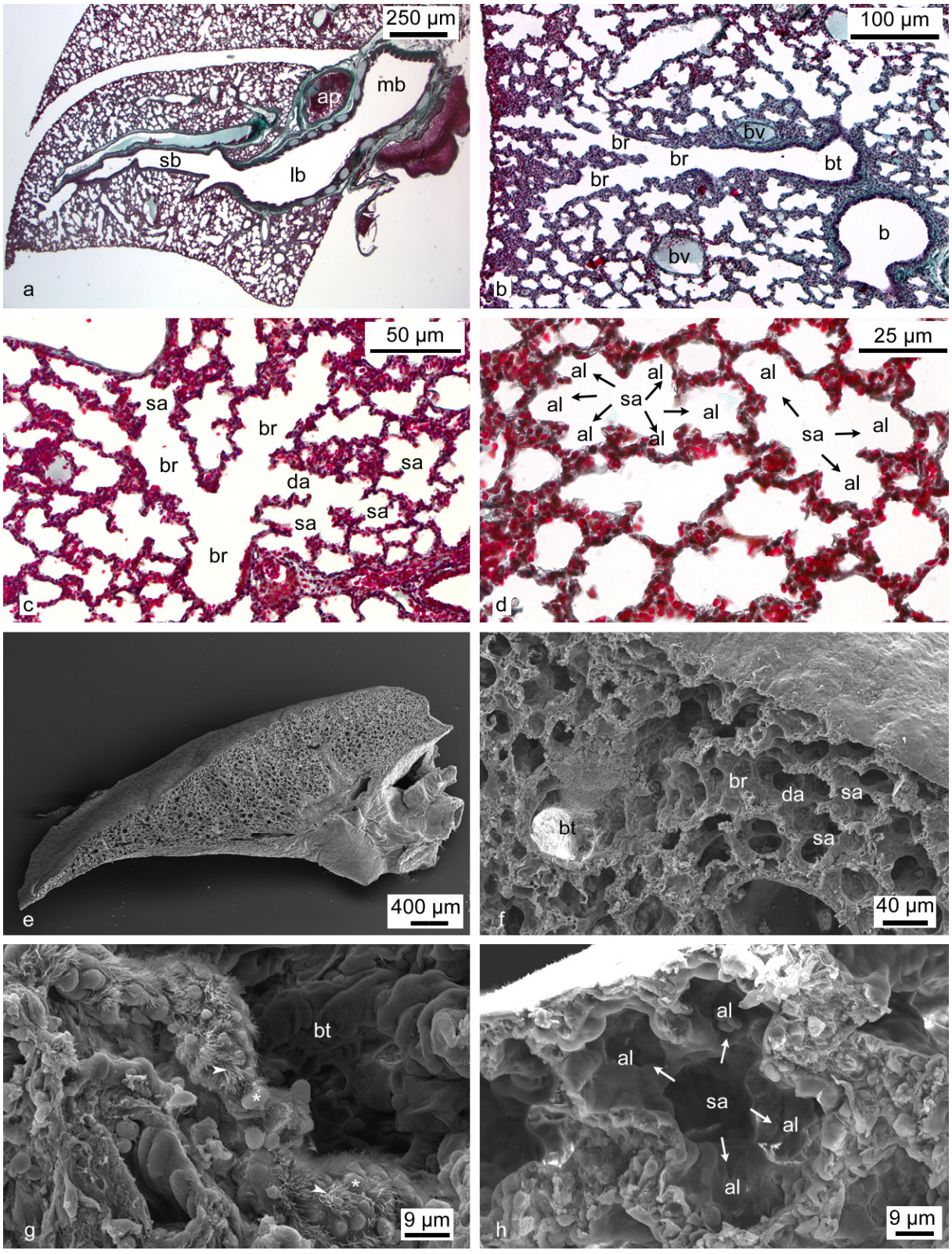

Fig. 78: Light micrographs (a-d) and scanning electron micrographs (e-h) of the lung of a seven days old *Tupaia belangeri*. The septation of the lung parenchyma has made further progress (a, e). Numerous new alveoli are formed in the distal portions of the airways and respiratory bronchioles and alveolar ducts become more distinct (b, c, f). Alveolar sacs with radiating alveoli are common (d, h). The distal portions of the terminal bronchioles are densely covered with cilia (g; arrowheads indicate cilia; asterisks show nonciliated cells). ap, pulmonary artery; al, alveole; b, bronchiole; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; da, alveolar duct; lb, lobar bronchus; mb, main bronchus; sa, alveolar sac; sb, segmental bronchus. Trichrome staining. Magnification is indicated by the scale bar.

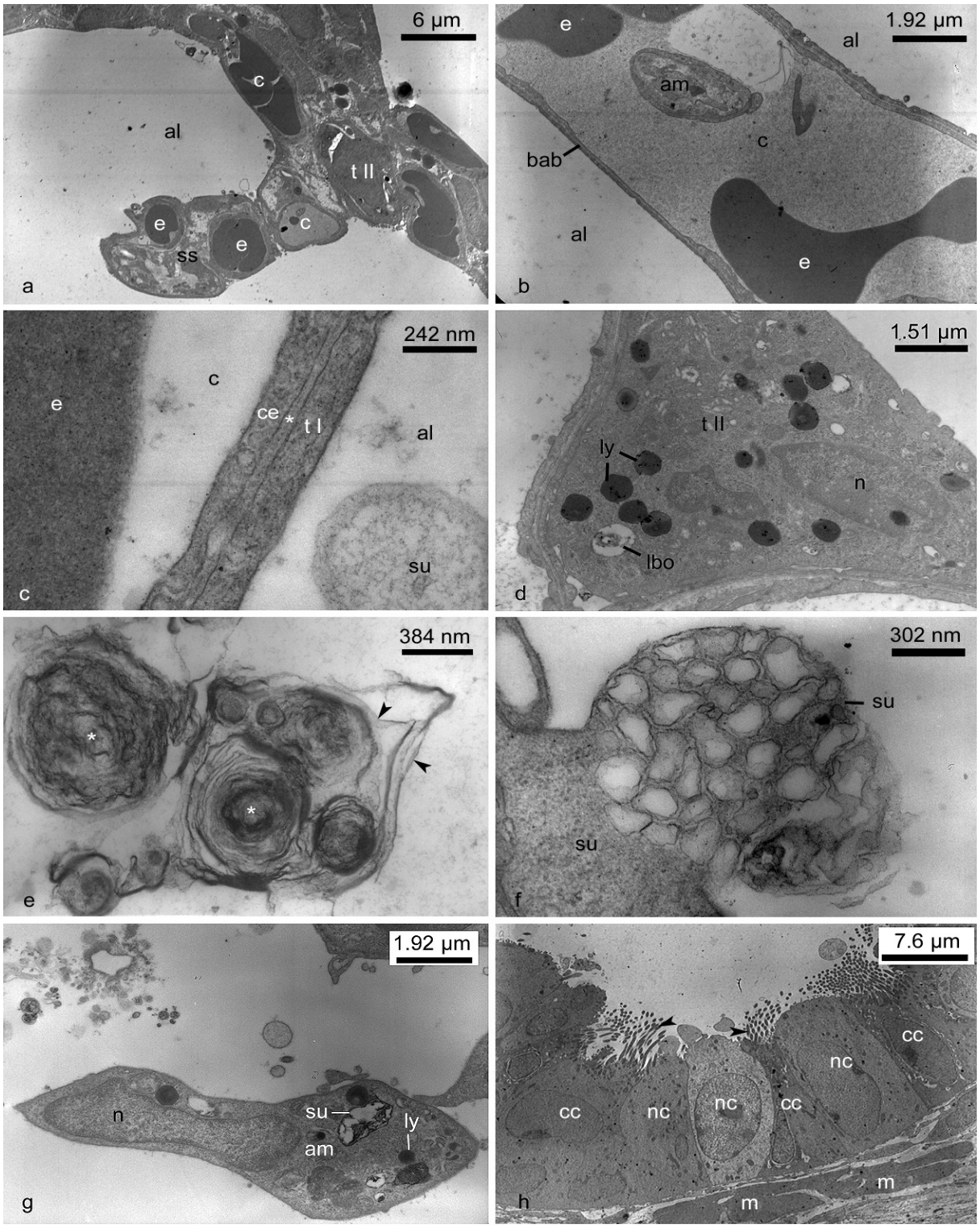

Fig. 79: Transmission electron micrographs of the lung of a seven days old *Tupaia belangeri.* Further septal outgrowths protrude into the air space and separate new alveoli (a). The septa contain a single capillary spanning the entire septum (b). The blood-air barrier resembles that of earlier developmental stages (c, asterisk shows basal lamina). Some type II pneumocytes contain lysosomes (d). The secretion of surfactant into the alveolar lumen proceeds and a lot of free surfactant is present. It transforms from lamellae (asterisk) into a thin film (arrowhead) (e) or a reticular form (f). Surplus surfactant is removed by alveolar macrophages (g). In the terminal bronchioles ciliated and non-ciliated cells alternate (h; arrowheads show cilia). al, alveole; am, alveolar macrophage; c, capillary; cc, ciliated cell; ce, capillary endothelium; e, erythrocyte; lbo, lamellar body; ly, lysosome; m, muscle cell; n, nucleus; nc, nonciliated cell; ss, single capillary septum; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

3.2.3 Precocial Placentalia

Based upon the histological examination of lung tissue from a variety of newborn mammals, Engel (1953) concluded that pulmonary development parallels the overall level of development at birth. Since the newborns of *Cavia aperea* and *Macroscelides proboscideus* are born at highly precocial conditions (see 3.1.1), also the lung structure of the neonates is advanced (Fig. 80).

3.2.3.1 Cavia aperea

The neonate of *Cavia aperea* is born at a highly precocial condition (see chapter 3.1.1). At birth, the neonate is fully furred, has open eyes and is able to walk and run. Also the internal organs, including the lung, are already mature. The advanced lung structure of the neonatal *Cavia aperea* is shown in figure 80 a.

The lung of the neonatal *Cavia aperea*

The newborn lung of *Cavia aperea* is at the alveolar stage of lung development. The lung parenchyma is highly subdivided and numerous alveoli and their associated structures,

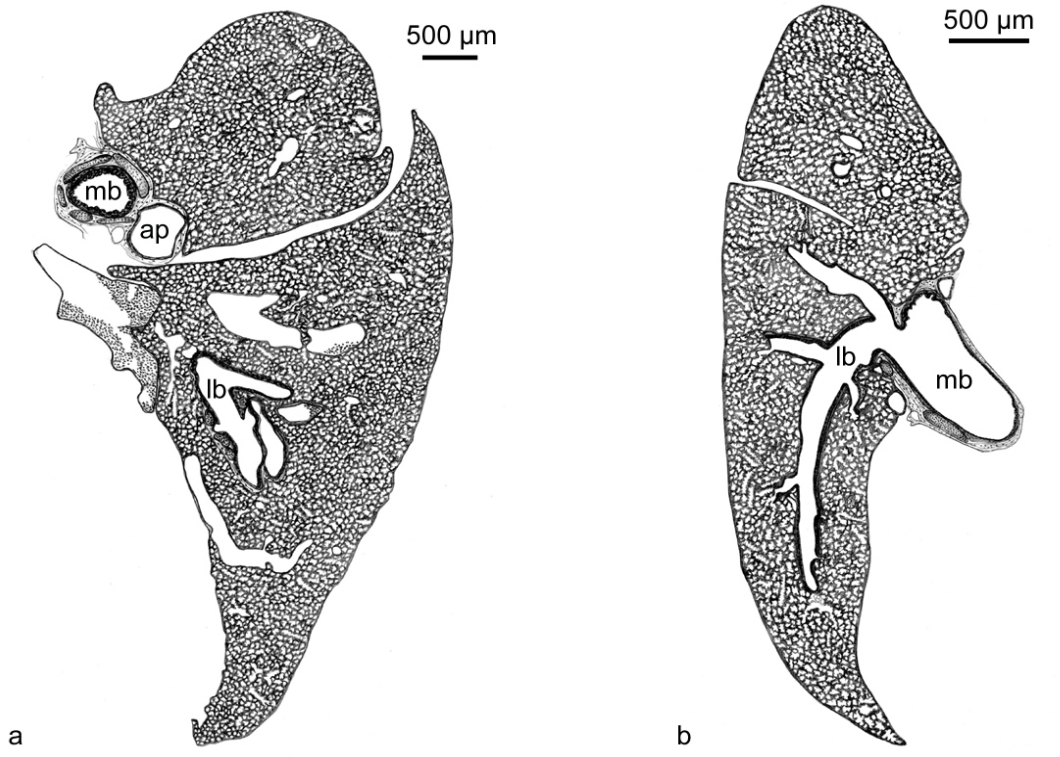

Fig. 80: Original drawings from histological sections of the left lung of a newborn *Cavia aperea* (a) and of the right lung of a newborn *Macroscelides proboscideus* (b). ap, pulmonary artery; lb, lobar bronchus; mb, main bronchus Magnification a: 25 x, b: 50 x.

such as respiratory bronchioles, alveolar ducts and alveolar sacs are already present (Fig. 82 a, b). A schematic reconstruction of the neonatal bronchial tree is shown in figure 81. The light microscopic and electron microscopic findings of the lung structure of the neonatal *Cavia aperea* are presented in figure 82. The size of the right and the left lung is nearly the same. The longitudinal expansion of the right lung is 16.0 mm, whereas the left lung measures 15.8 mm in total length. Relating to the CRL of 80 mm, the right lung takes a fifth part of the whole body length.

The right lung of *Cavia aperea* consists of completely separated superior, middle, accessory and inferior lobes. The left lung is formed of middle, accessory and inferior lobes. The branching pattern of the lobar bronchi indicates the several regions of the lungs (Fig. 81 b). In the right lung, the superior lobe bronchus is formed by the first lateral branch from the main bronchus. The middle lobe is supplied by the second bronchus, which branches off from the ventro-lateral side of the right main bronchus. The accessory lobe bronchus arises from the medio-ventral side of the right main bronchus. The other following bronchi supply the inferior lobe of the right lung. In the left lung, the first branch of the lateral side of the left main bronchus supplies the middle lobe. *Cavia aperea* has an

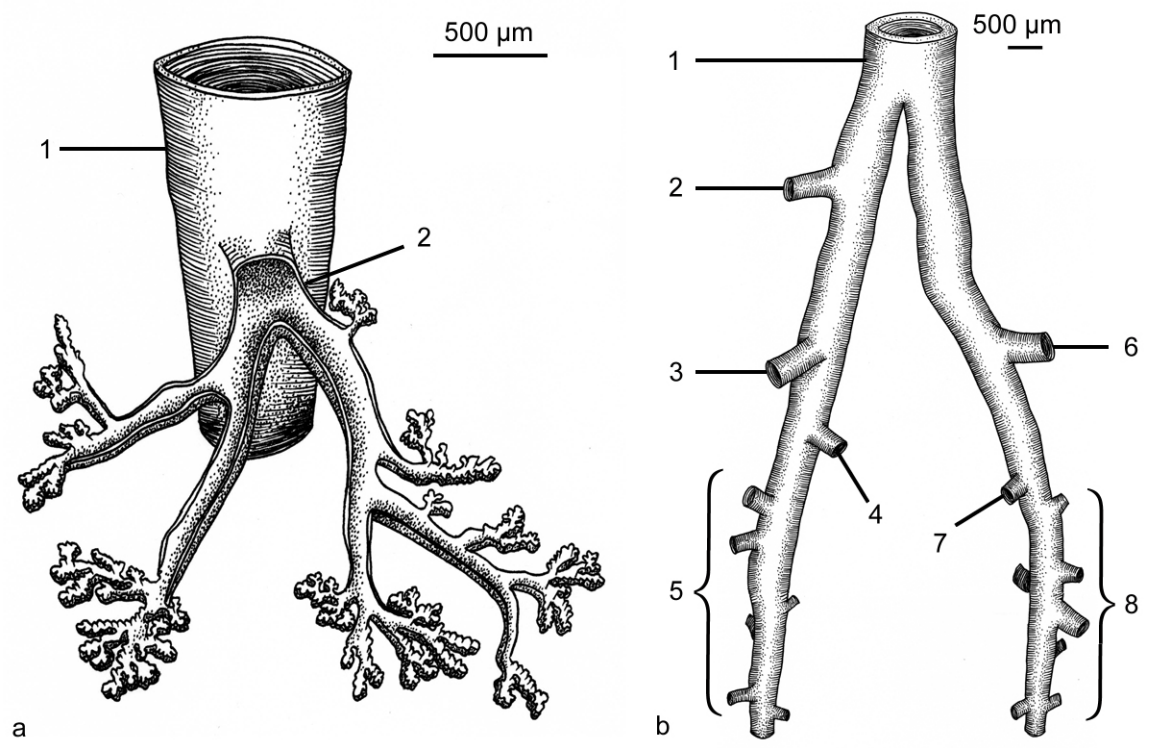

Fig. 81: Reconstruction and schematic representation of the main bronchus of the left lung with middle lobe bronchus branching off (a) and of the bronchial tree (b) of the lung of a neonatal *Cavia aperea*. a: 1 – main bronchus; 2 – middle lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchus; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 - middle lobe bronchus; 7 – accessory lobe bronchus; 8 – inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

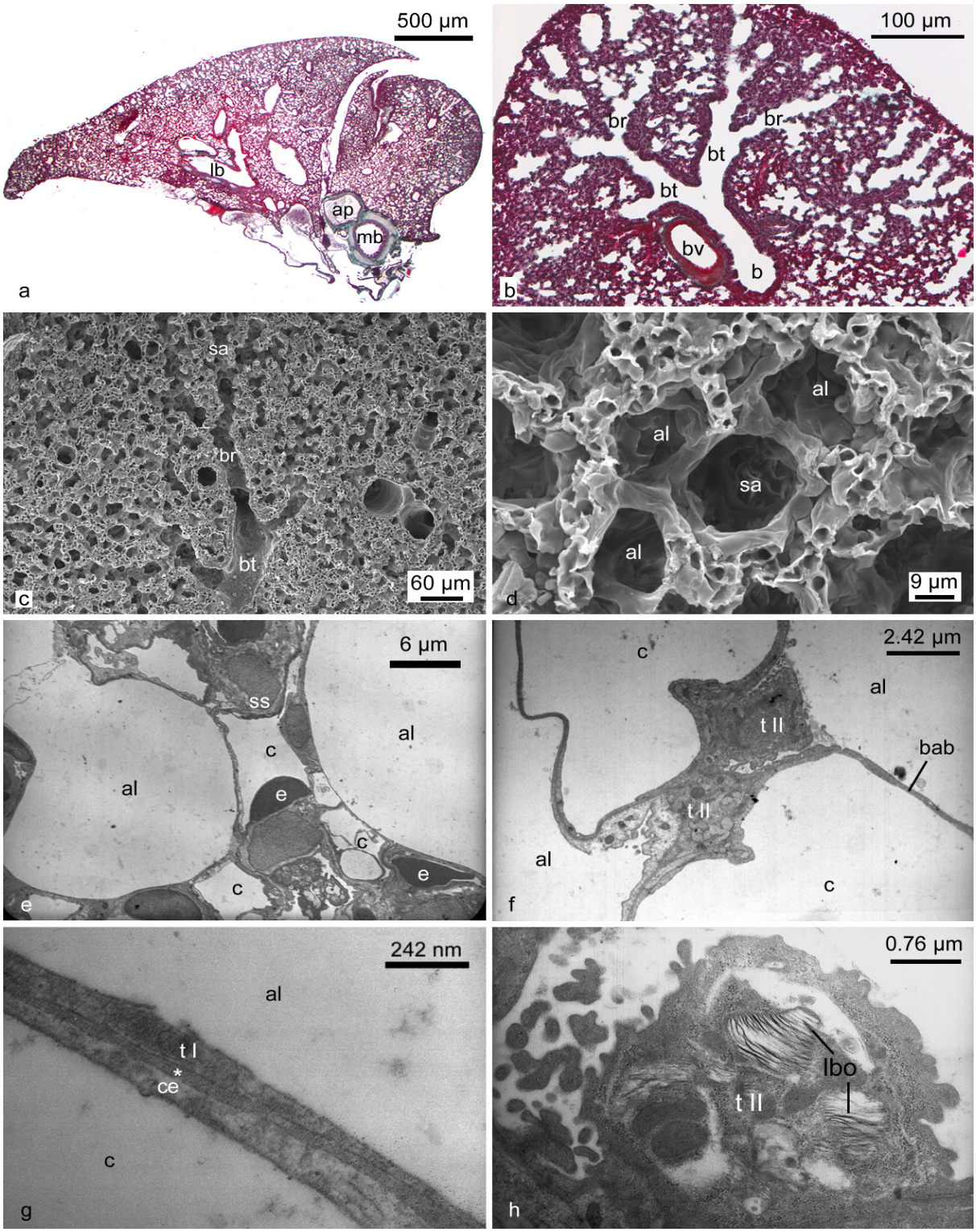

Fig. 82: Light micrographs (a, b) and electron micrographs (c-h) of the neonatal lung of *Cavia aperea*. The lung is at the alveolar stage and is highly subdivided (a). In the distal portions of the bronchiolar system respiratory bronchioles are already present (b, c). In the terminal portion of the airways numerous small alveoli radiate from alveolar sacs in a concentric way (d). The alveoli are separated by single capillary septa (e). These are characterised by a single capillary spanning the entire septum (f). The trilaminar blood-air barrier is thin (g; basal lamina indicated by asterisk). The cuboidal type II pneumocytes contain large lamellar bodies (h). ap, pulmonary artery; al, alveole; b, bronchiole; bab, blood-air barrier; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; c, capillary; ce, capillary endothelium; e, erythrocyte; lb, lobar bronchus; lbo, lamellar body; mb, main bronchus; sa, alveolar sac; ss, single capillary septum; t I, type I pneumocyte; t II, type II pneumocyte. Trichrome staining. Magnification is indicated by the scale bar.

accessory lobe bronchus not only in the right lung, but also in the left lung. This bronchus branching off from the medio-ventral side of the left main bronchus supplies an independent pulmonary lobe. The accessory lobe is smaller in the left lung than in the right. The inferior lobe of the left lung is supplied by several bronchi branching off from the distal part of the left main bronchus.

The bronchial tree is well developed and leads with many dichotomies deep into the periphery of the lung. The main bronchi measure 750 µm in diameter in the proximal parts and approximately 400 µm in diameter in the more distal parts. They are lined with two- to three-layered cuboidal ciliated epithelium. Beneath a thick layer of four to five smooth muscle cells is lying. The main bronchi are supported by cartilage extra- and intrapulmonar. The cartilage extends up to the distal portions of the main bronchi. Loose connective tissue encircles the cartilage and submucosal glands and separates the main bronchi from the surrounding lung parenchyma. The lobar bronchi, branching off from the main bronchi, measure approximately 300-400 µm in diameter. They are lined with one-layered columnar ciliated epithelium. Beneath a layer of two to three smooth muscle cells follows. Similar to the main bronchi, the walls of the lobar bronchi are supported by cartilage. The larger bronchioles of the newborn lung measure up to 100 µm in diameter. They are lined with one-layered cuboidal epithelium. The bronchiolar walls are supported by a thin layer of one to two smooth muscle cells. The terminal portions of the airways consist of terminal and respiratory bronchioles (Fig. 82 b, c). The terminal bronchioles measure approximately 50-60 µm in diameter, whereas the respiratory bronchioles are smaller with only 30-40 µm in diameter. In the distal portions of the terminal bronchioles the epithelium is grading first into low cuboidal and ultimately into squamous epithelium in the form characteristic for alveolar membranes. Alveoli are evident in these bronchiolar walls, what refers to typical respiratory bronchioles. The respiratory bronchioles pass into short alveolar ducts, which open into alveolar sacs. The alveolar sacs measure approximately 60 µm in diameter. From the centre of the alveolar sacs several small alveoli radiate in a concentric way (Fig. 82 d).

The numerous alveoli are small in size. They measure only 15-20 µm in diameter. The alveolar epithelium consists of squamous type I pneumocytes and occasionally interspersed cuboidal type II pneumocytes. The type II pneumocytes are similar to that found in other mammalian species. They contain some large lamellar bodies, which synthesise, store and secrete phospholipids in form of surfactant (Fig. 82 h).

The alveoli are formed by sprouting from the alveolar sacs into the surrounding lung parenchyma and are separated by septal outgrowths. In the lung of the newborn *Cavia aperea* only single capillary septa are present (Fig. 82 e, f). Wide capillaries situated in the centre of the septum or alternately protruding at each side of a thin axis of connective tissue components form a single capillary network. The alveolar septa have a thickness of 7-9 µm.

The blood-air barrier has a trilaminar structure. It is composed of an epithelial layer with type I pneumocytes, an endothelial layer with capillary endothelial cells and a fused basal lamina of both cell types (Fig. 82 g). The diffusion barrier measures only 200-250 µm in thickness.

The lung of the four days old *Cavia aperea*

On the whole, the four days old lung resembles that of the newborn *Cavia aperea*. However, in the four days old lung the lung parenchyma appears to be less compact than in the newborn lung (Fig. 83 a, c). The light microscopic and electron microscopic findings of the lung of a four days old *Cavia aperea* are shown in figure 83.

Compared to the newborn lung, the bronchial tree of the four days old lung shows no structural changes. With the proceeding formation of alveoli, in the distal portions of the airways respiratory bronchioles and alveolar ducts become more evident (Fig. 83 a, b).

The less compact appearance of the lung parenchyma of the four days old lung is caused by a size increase of the alveoli. The alveoli measure now 20-30 µm in diameter. They are separated from each other by septa with a single capillary spanning the entire septum (Fig. 83 e). Alveoli are clearly recognisable at the walls of the respiratory bronchioles and alveolar ducts (Fig. 83 b), but mainly they are radiating from the centre of alveolar sacs (Fig. 83 d). With approximately 70 µm in diameter, also the alveolar sacs become slightly larger.

In the lung of the four days old *Cavia aperea* the synthesis of surfactant proceeds. The type II pneumocytes produce a lot of surfactant inside their large lamellar bodies and secrete it into the alveolar lumen (Fig. 83 f). Some surfactant can be found free in the air space or lining the alveolar epithelium.

The lung of the adult *Cavia aperea*

The overall appearance of the lung parenchyma in the adult *Cavia aperea* is highly sub-divided (Fig. 84 a). The striking findings of the adult lung are the surface enlargement of the gas exchanging structures and the thinning of the septa. The light microscopic and electron microscopic findings of the adult lung of *Cavia aperea* are presented in the figures 84 and 85.

The bronchial tree is getting longer in consequence of the overall size increase of the lung. The main bronchi measure 850 µm in diameter in the proximal parts. They are lined with two- to three-layered cuboidal ciliated epithelium. Beneath a thick layer or partly patches of five to seven smooth muscle cells are lying. Cartilage is present in the walls of main, lobar and segmental bronchi. The walls of the lobar and segmental bronchi are lined by one-layered columnar epithelium. Their walls contain a thick circular layer of five to seven smooth muscle

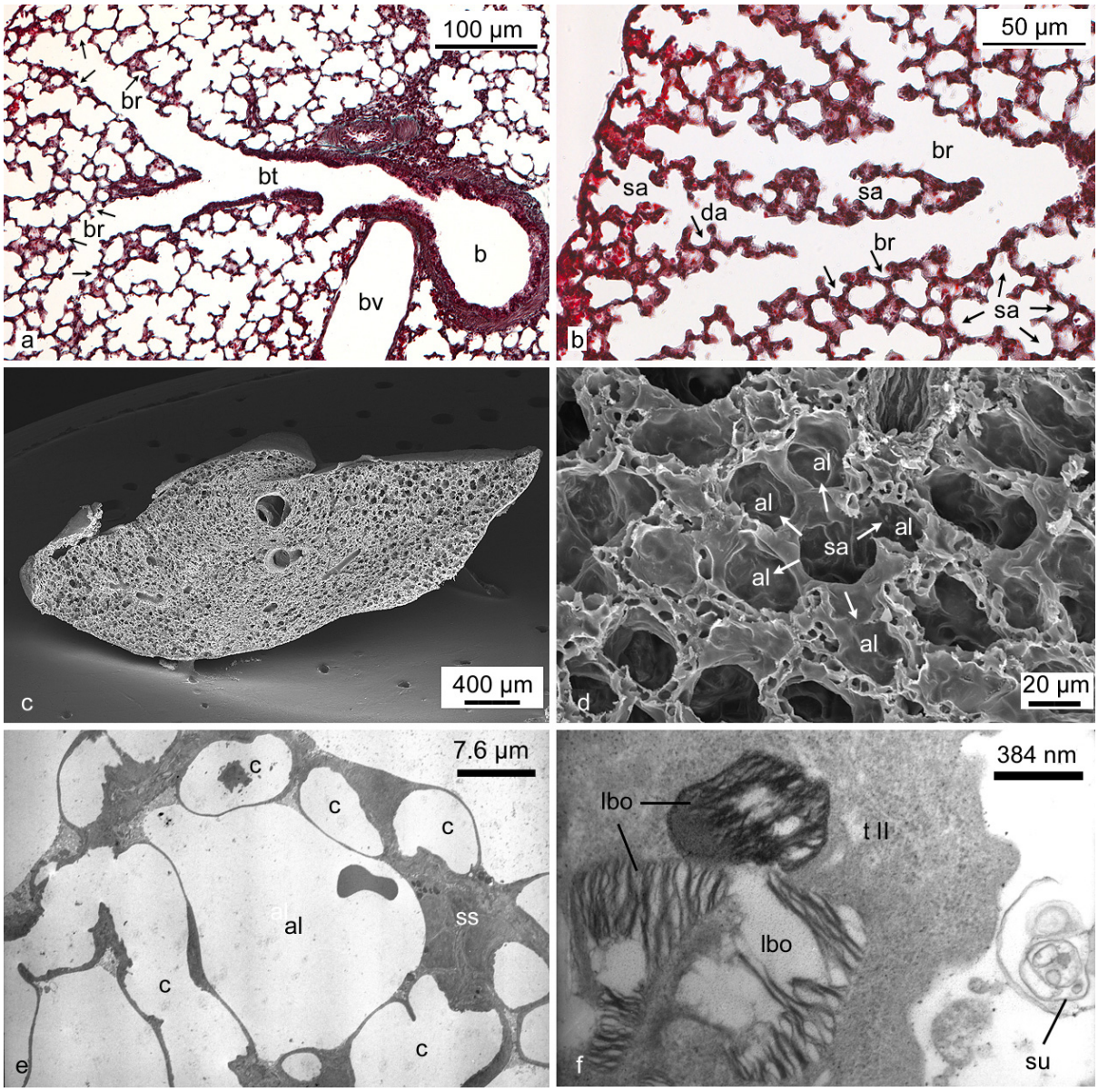

Fig. 83: Light micrographs (a, b) and electron micrographs (c-f) of the lung of a 4 days old *Cavia aperea*. In the four days old lung the lung parenchyma appears to be less compact than in the newborn lung (a, c). The alveoli become larger and respiratory bronchioles, alveolar ducts and alveolar sacs are more distinct (b, d; alveoli are indicated by arrowheads). The alveoli are separated by septa with a single capillary spanning the septum (e). The lamellar bodies of the type II pneumocytes synthesise plenty of phospholipids and a lot of surfactant can be found free in the alveolar lumen (f). al, alveole; b, bronchiole; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; c, capillary; da, alveolar duct; lbo, lamellar body; sa, alveolar sac; ss, single capillary septum; su, surfactant; t II, type II pneumocyte. Trichrome staining. Magnification is indicated by the scale bar.

cells. The contraction of the massive muscular layer causes longitudinal folds in the bronchial epithelium. The bronchioles of the adult lung resemble that of the newborn lung. However, with a layer of two to three smooth muscle cells, they are more muscular. In the distal portions of the terminal bronchioles ciliated cells can be found. These ciliated cells are characterised by cilia protruding from the apical region of the cell (Fig. 85 d). Their function is to move trapped airborne particles and secretions toward the pharynx. In the distal portions,

the terminal bronchioles give rise to respiratory bronchioles, which pass into alveolar ducts. The respiratory bronchioles as well as the alveolar ducts are characterised by a high degree of alveolarisation in their walls (Fig. 84 b).

The short alveolar ducts open into alveolar sacs, from which alveoli radiate in a concentric way (Fig. 84 c, d). The septal crests, which separate the alveoli, become longer and lead partly to a subseptation of the alveolar sacs. Furthermore new alveoli sprout from older alveoli into the surrounding parenchyma and lead to a surface enlargement of the gas exchanging structures of the adult lung (Fig. 85 a). The single capillary septa, which separate the alveoli from each other, become thinner and measure now 6 µm in thickness (Fig. 85 b). The blood-air barrier resembles that of the newborn lung (Fig. 85 c). The trilaminar structure and the thickness of the diffusion barrier are the same. In the endothelial layer of the blood-air barrier numerous coated vesicles are present. These vesicles are involved in the

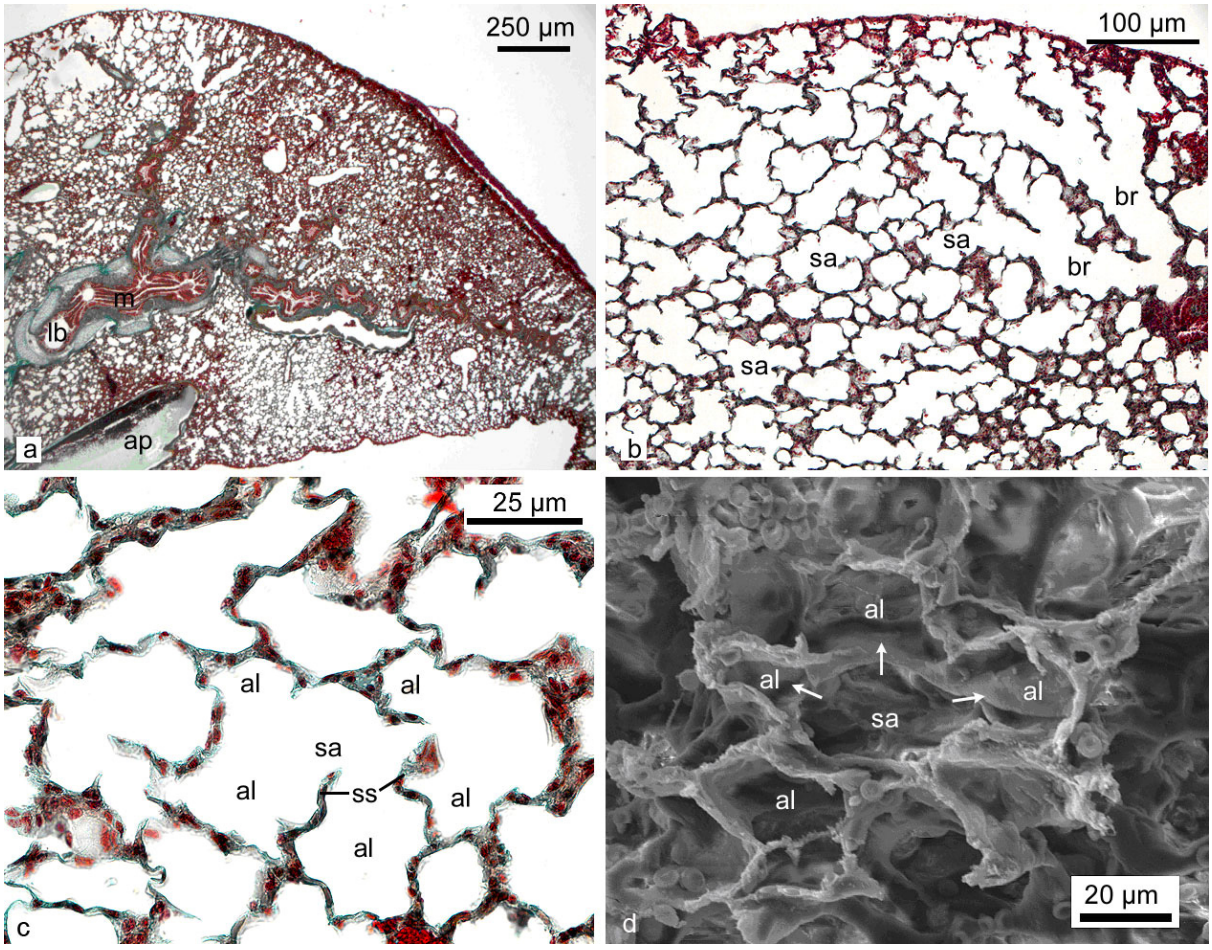

Fig. 84: Light micrographs (a-c) and scanning electron micrographs (d) of the adult lung of *Cavia aperea*. The septation of the lung parenchyma has made further progress (a). Numerous new alveoli are formed in the distal portions of the airways and respiratory bronchioles and alveolar ducts are more numerous (b; alveoli are indicated by arrowheads). The alveolar sacs increase in size (c, d) and the septal outgrowths separating the alveoli become longer. ap, pulmonary artery; al, alveole; b, bronchiole; br, respiratory bronchiole; da, alveolar duct; lb, lobar bronchus; m, smooth musculature; sa, alveolar sac; ss, single capillary septum. Trichrome staining. Magnification is indicated by the scale bar.

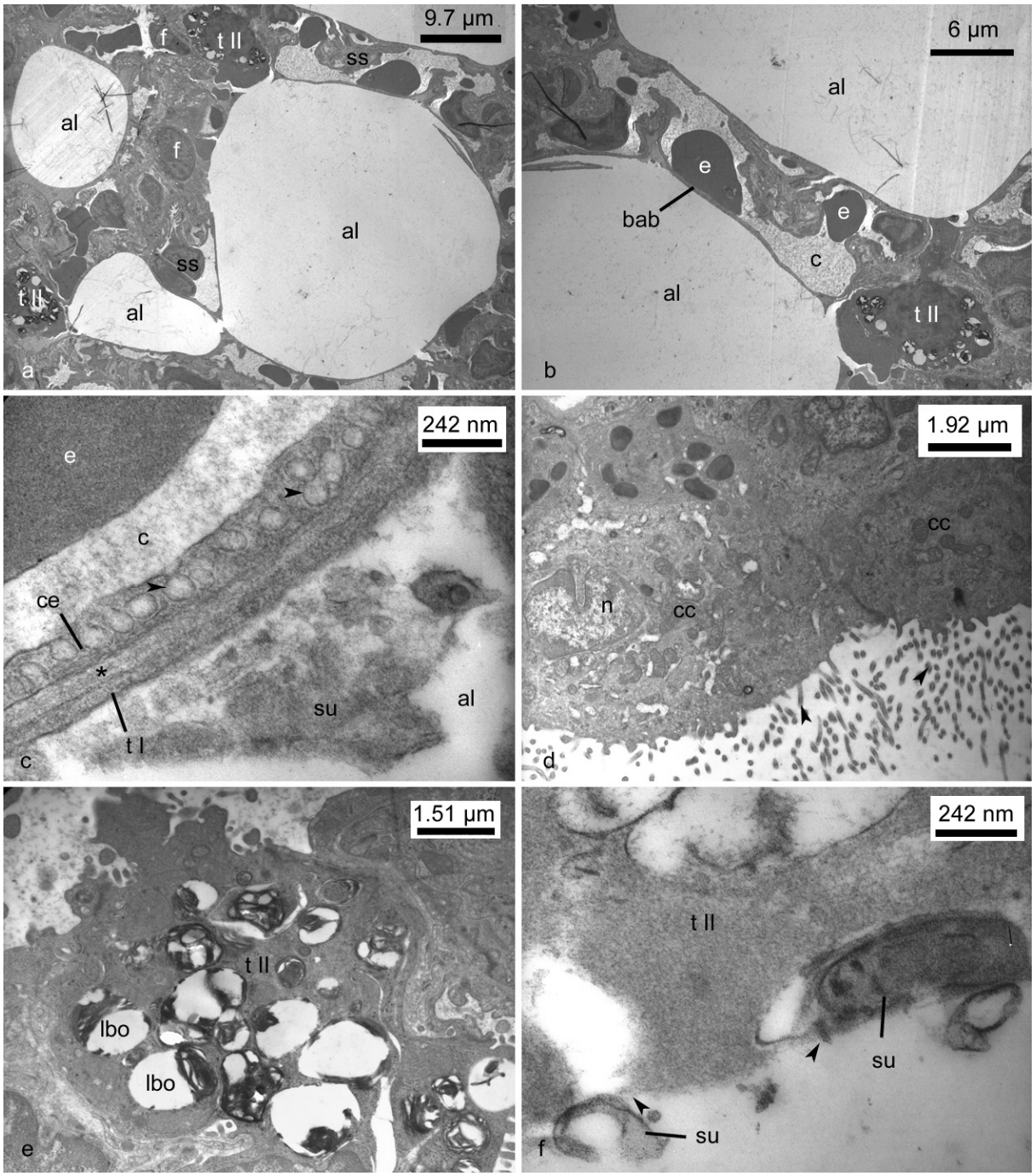

Fig. 85: Transmission electron micrographs of the lung of a adult *Cavia aperea.* From older alveoli, new alveoli sprout into the surrounding lung parenchyma (a). The alveoli are separated by septal outgrowths. The single capillary septa resemble that of earlier developmental stages, however they become slightly thinner (b). The blood-air barrier consists of capillary endothelial cells, alveolar epithelial cells and a fused basal lamina (∗) of both cell types (c, arrowheads show coated vesicles). The type II pneumocytes contain many lamellar bodies, which synthesise surfactant (e). The secretion of surfactant into the alveolar lumen proceeds (f, arrowheads indicate the exocytosis of surfactant). In the terminal bronchioles ciliated cells are present (h; arrowheads show cilia). al, alveole; bab, blood-air barrier; c, capillary; cc, ciliated cell; ce, capillary endothelium; e, erythrocyte; f, fibroblast; lbo, lamellar body; n, nucleus; ss, single capillary septum; su, surfactant; t I, type I pneumocyte; t II, type II pneumocyte. Magnification is indicated by the scale bar.

transport of proteins. A fourth component of the blood-air barrier can be distinguished. Surfactant covers the apical surface of the type I pneumocytes.

The surfactant is produced in the type II pneumocytes. As surfactant is synthesised, it is sequestered within granules in a lipid bilayer lamellar form. Lamellae continue to accumulate as surfactant is added to the granule. Compared to the newborn lung, the type II pneumocytes of the adult lung contain many lamellar bodies (Fig. 85 e). These lamellar bodies release their content by exocytosis (Fig. 85 f). During this process the lamellae unfold and transform into a thin film, which is the primary surface-active component. This film lines the alveolar epithelium in order to reduce the surface tension of the air spaces.

3.2.3.2 Macroscelides proboscideus

Macroscelides proboscideus is born at a highly precocial condition (see chapter 3.1.1). It is fully furred, the eyes are open and although it is philopatric, it is able to coordinate locomotion. According to the advanced external characters, also the internal organs are mature at birth. The advanced lung structure of the neonatal *Macroscelides proboscideus* is presented in figure 80 b.

The lung of the neonatal *Macroscelides proboscideus*

The newborn lung of *Macroscelides proboscideus* is at the alveolar stage of lung development. The lung parenchyma is highly subdivided and long respiratory bronchioles are predominant in the lung (Fig. 87 a, c). A schematic reconstruction of the neonatal bronchial tree is shown in figure 86. The light microscopic and electron microscopic findings of the lung structure of the neonatal *Macroscelides proboscideus* are presented in figure 87.

The right lung is slightly larger than the left one. The longitudinal expansion of the right lung is 7.0 mm, whereas the left lung measures 6.0 mm in total length. Relating to the total body length of 60 mm, the right lung takes an eighth of the whole body length. The right lung of *Macroscelides proboscideus* consists of completely separated superior, middle, accessory and inferior lobes. The left lung is formed of superior, middle and inferior lobes. All pulmonary lobes of both lungs have incompletely fissures, which indicate a further subdivision of the lobes. The branching pattern of the lobar bronchi indicates the several regions of the lungs (Fig. 86 b). In the right lung, the superior lobe is supplied by the first three lateral branches from the main bronchus. The middle lobe bronchus is formed by a large bronchus branching off from the ventral side of the right main bronchus. The next bronchus, which arises from the medial side of the right main bronchus, supplies the accessory lobe. All other following bronchi form the inferior lobe bronchi of the right lung. In the left lung, the first branch of the

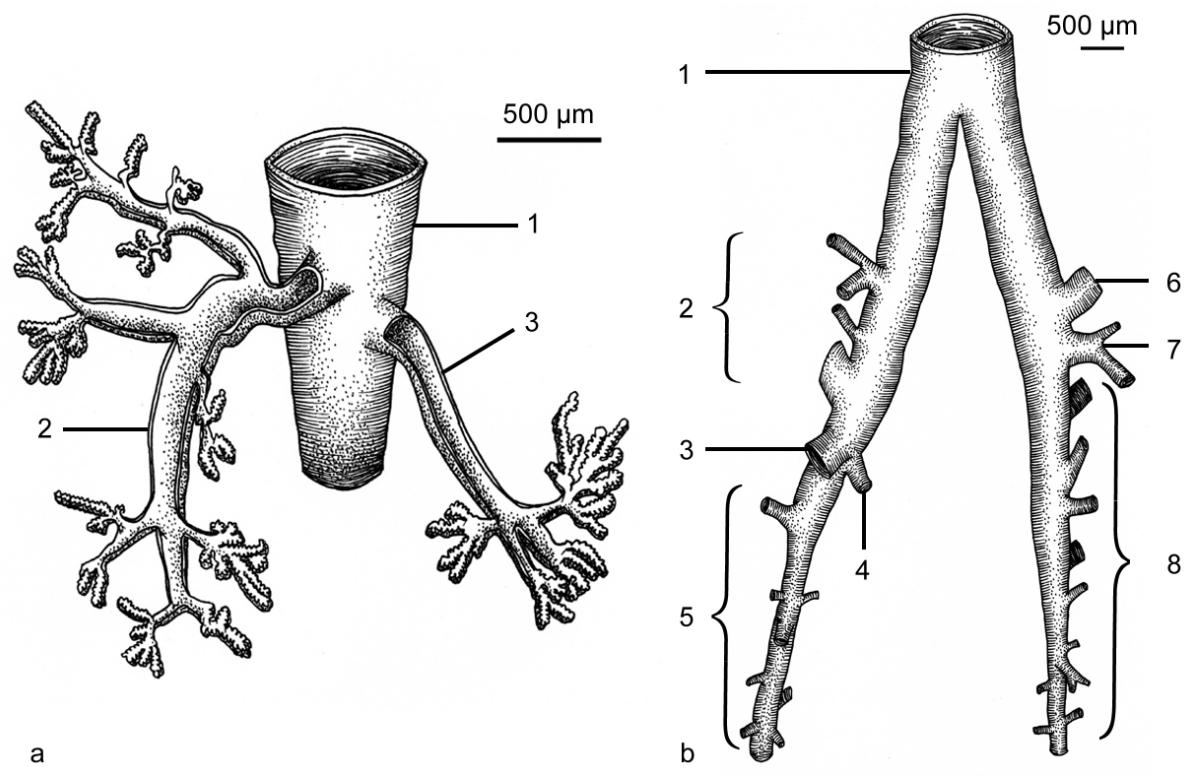

Fig. 86: Reconstruction and schematic representation of the main bronchus of the right lung with middle lobe and accessory lobe bronchi branching off (a) and of the bronchial tree (b) of the lung of a neonatal *Macroscelides proboscideus*. a: 1 – main bronchus; 2 – middle lobe bronchus; 3 – accessory lobe bronchus. b: 1 - trachea; right lung: 2 – superior lobe bronchi; 3 – middle lobe bronchus; 4 – accessory lobe bronchus; 5 – inferior lobe bronchi; left lung: 6 - superior lobe bronchus; 7 – middle lobe bronchus; 8 – inferior lobe bronchi. Magnification a: 50 x; b: 25 x.

lateral side of the left main bronchus supplies the superior lobe. The middle lobe bronchus is formed by the second branch from the lateral side of the left main bronchus. The numerous following bronchi supply the large inferior lobe of the left lung. The bronchial tree of the neonatal *Macroscelides proboscideus* is well developed and extends with many dichotomies to the periphery of the lung (Fig. 87 b). The main bronchi measure 600 µm in diameter in the proximal parts and approximately 300 µm in diameter in the more distal parts. They are lined with one-layered cuboidal ciliated epithelium. Beneath a layer of three to four smooth muscle cells is lying. The main bronchi are supported by cartilage only extrapulmonar up to the second dichotomy. Loose connective tissue encircles the cartilage and submucosal glands and separates the main bronchi from the surrounding. The lobar bronchi measure 250-300 µm in diameter. The lining epithelium is the same as in the main bronchi, one-layered cuboidal ciliated epithelium. The bronchial walls are supported only by a layer of two to three smooth muscle cells, no cartilage is present. The larger bronchioles measure 100-150 µm in diameter. They are lined with simple cuboidal epithelium and have a thin layer of one to two smooth muscle cells in their walls. The terminal airways consist of terminal bron-chioles with a diameter of 70-80 µm and respiratory bronchioles with a diameter of 50-60 µm (Fig. 87 c,

d). The extremely long respiratory bronchioles are highly alveolarised and may have a big part in the total gas exchange of the newborn lung. In the distal portions, the respiratory

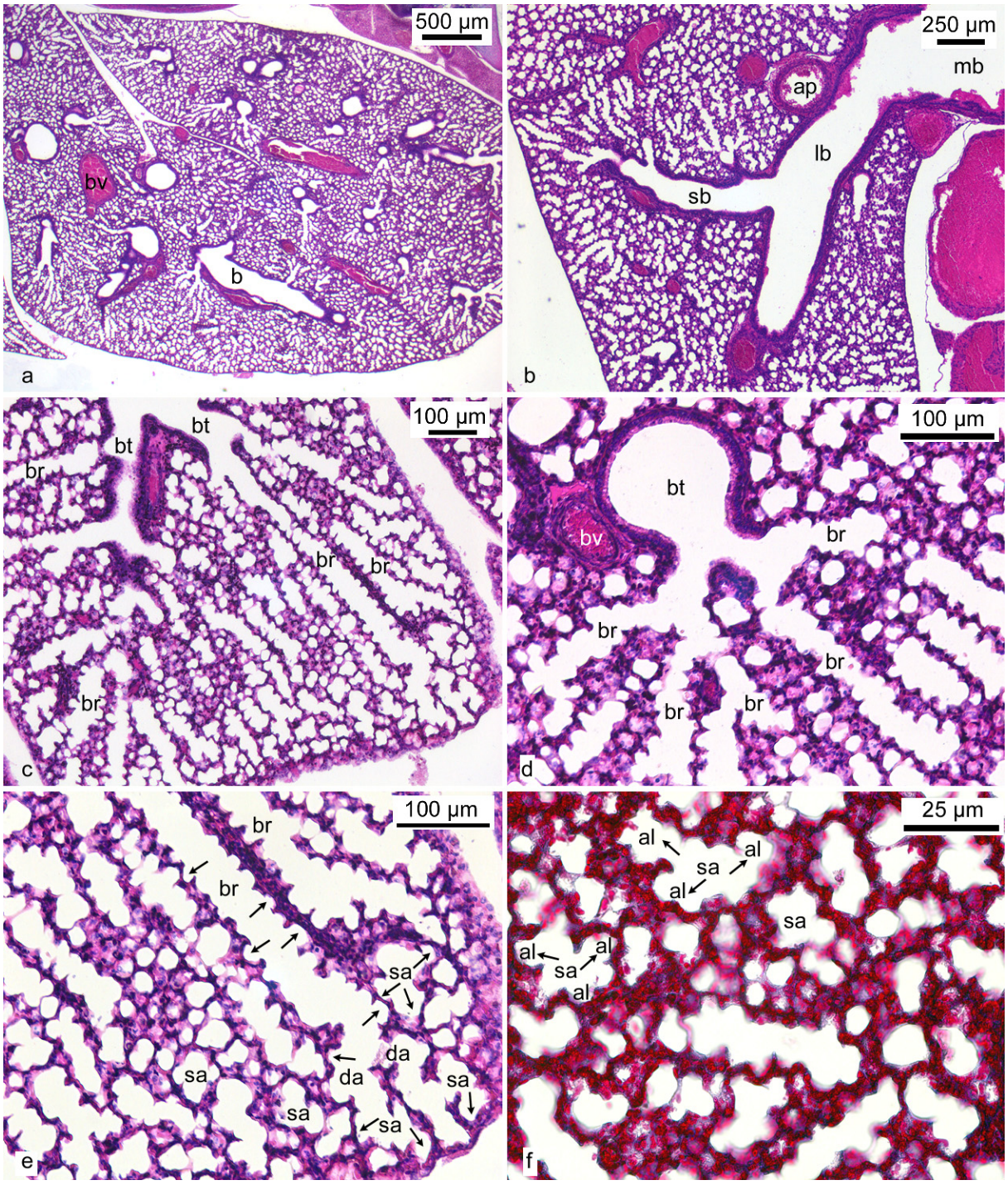

Fig. 87: Light micrographs of the newborn lung of *Macroscelides proboscideus*. At birth, the lung parenchyma is highly subdivided (a). The bronchial tree is well developed and extends with many dichotomies far into the periphery of the lung (b). In the distal portions of the bronchiolar system long respiratory bronchioles and alveolar ducts are already present (c, d, e; alveoli are indicated by arrows). The numerous small alveoli radiate from small alveolar sacs (f). al, alveole; ap, pulmonary artery; b, bronchiole; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; da, alveolar duct; lb, lobar bronchus; mb, main bronchus; sa, alveolar sac; sb, segmental bronchus HE staining. Magnification is indicated by the scale bar.

bronchioles give rise to short alveolar ducts, which open into alveolar sacs (Fig. 87 e). The alveolar sacs are relatively small and measure only 30-40 µm in diameter. From the centre of the alveolar sacs several small alveoli of approximately 10-15 µm in diameter radiate into the surrounding lung parenchyma (Fig. 87 f). The septa separating the alveoli have a single capillary bed. They have a thickness of 5 µm.

The lung of the adult *Macroscelides proboscideus*

The striking findings of the adult lung are the surface enlargement of the gas exchanging structures and the size increase of alveoli and alveolar sacs. The septation of the lung parenchyma has made further progress (Fig. 88 a). The light microscopic findings of the adult lung of *Macroscelides proboscideus* are presented in the figure 88. The bronchial tree of the adult *Macroscelides proboscideus* resembles that of the newborn lung. The main and lobar bronchi and the bronchioles have nearly the same diameters and compositions of the walls as already described for the neonatal lung. There are no structural changes apparent. The thick muscular layer in the walls of the main bronchi causes longitudinal folds in the epithelial layer (Fig. 88 b). In the distal portions of the terminal airways respiratory bronchioles and alveolar ducts are common (Fig. 88 c, d, e). However, the extremely long respiratory bronchioles of the newborn lung are missing in the adult lung. Compared to the neonatal lung, the alveolar sacs of the adult lung of *Macroscelides proboscideus* are larger in size. They measure 50-60 µm in diameter and are often interconnected with each other (Fig. 88 f). The numerous alveoli, which radiate from the centre of the alveolar sacs, become larger as well. They have a size of 15-20 µm in diameter. The alveoli are separated from each other by septa with a single capillary network (Fig. 88 f, arrowheads). Compared to the newborn lung, the septa seem to become thinner in the adult lung. In connexion with the size increase of the alveoli and alveolar sacs the lung parenchyma of the adult lung appears less compact.

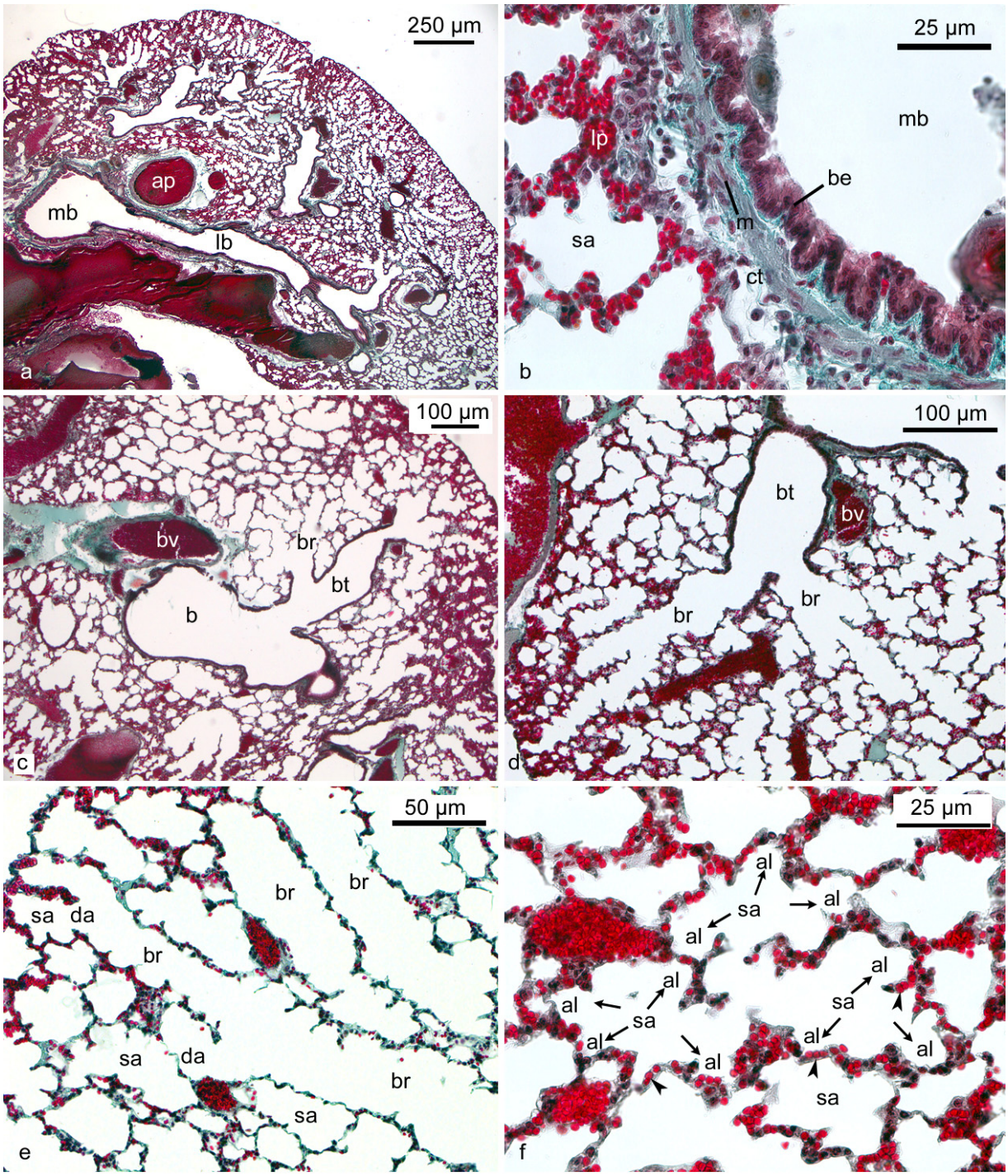

Fig. 88: Light micrographs of the adult lung of *Macroscelides proboscideus.* The septation of the lung parenchyma has made further progress (a). In the distal portions of the terminal airways respiratory bronchioles and alveolar ducts are common (c, d, e). The alveolar sacs increase in size and are frequently interconnected with each other (f; arrowheads indicate single capillary beds of septa). al, alveole; ap, pulmonary artery; b, bronchiole; be, bronchial epithelium; br, respiratory bronchiole; bt, terminal bronchiole; bv, blood vessel; ct, connective tissue; da, alveolar duct; lb, lobar bronchus; lp, lung parenchyma; m, muscle cell; mb, main bronchus; sa, alveolar sac. Trichrome staining. Magnification is indicated by the scale bar.

3.2.4 Monotremata (*Ornithorhynchus anatinus, Tachyglossus aculeatus*)

The order Monotremata of the mammalian subclass Prototheria is made up of two families, the Ornithorhynchidae and the Tachyglossidae. One aspect of their reproductive biology, that really sets them apart from all other mammals, is the fact that the females lay eggs and do not give birth to their young. From the Hill collection two pouch young monotremes were available for comparative investigations of the lung. A survey of the overall developmental degree and of the lung structure of *Ornithorhynchus anatinus* and *Tachyglossus aculeatus* give the figures 89 and 90.

Temple-Smith and Grant (2001) discuss a gestation period of 15-21 days for *Ornithorhynchus anatinus* and of 21-23 days for *Tachyglossus aculeatus*. After that time one to three eggs in *Ornithorhynchus anatinus* and a single egg in *Tachyglossus aculeatus* is layed by the female (Puschmann, 2004). After an incubation period of approximately ten days in both species the highly altricial neonates hatch. The young monotreme which hatches from the egg is very similar to the young marsupial in its stage of development and is maintained on milk from the mother either as a pouch young, in the case of the *Tachyglossus aculeatus,* or as a nestling in a burrow in the case of *Ornithorhynchus anatinus* (Griffiths, 1978; Grant, 1995). The newly hatched young of *Tachyglossus aculeatus* has a CRL of 15 mm and weighs 0.3-0.4 g, and it is probable that the young of *Ornithorhynchus anatinus* are of a similar size at hatching (Grant, 1995, Puschmann, 2004).

The age of the two pouch young examined from the Hill collection were not specified. The pouch young of *Tachyglossus aculeatus* has a length of 33 mm. The pouch young has

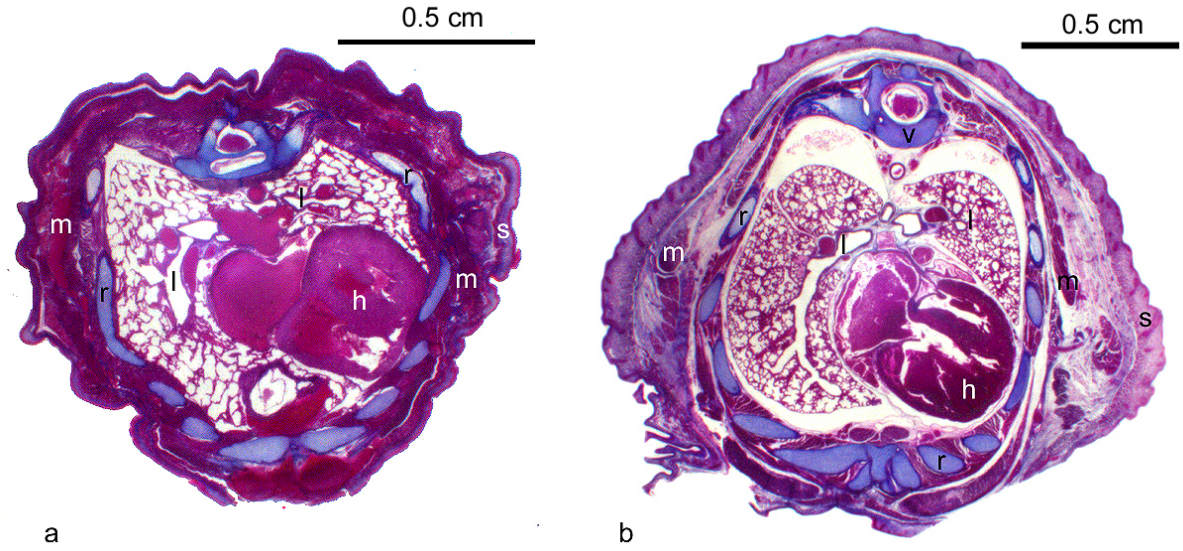

Fig. 89: Light micrographs of histological sections through the anterior region of a pouch young *Ornithorhynchus anatinus* (a) and a pouch young *Tachyglossus aculeatus* (b). h, heart; l, lung; m, musculature; r, rib; s, skin; v, vertebra. Azan staining. Magnification is indicated by the scale bar.

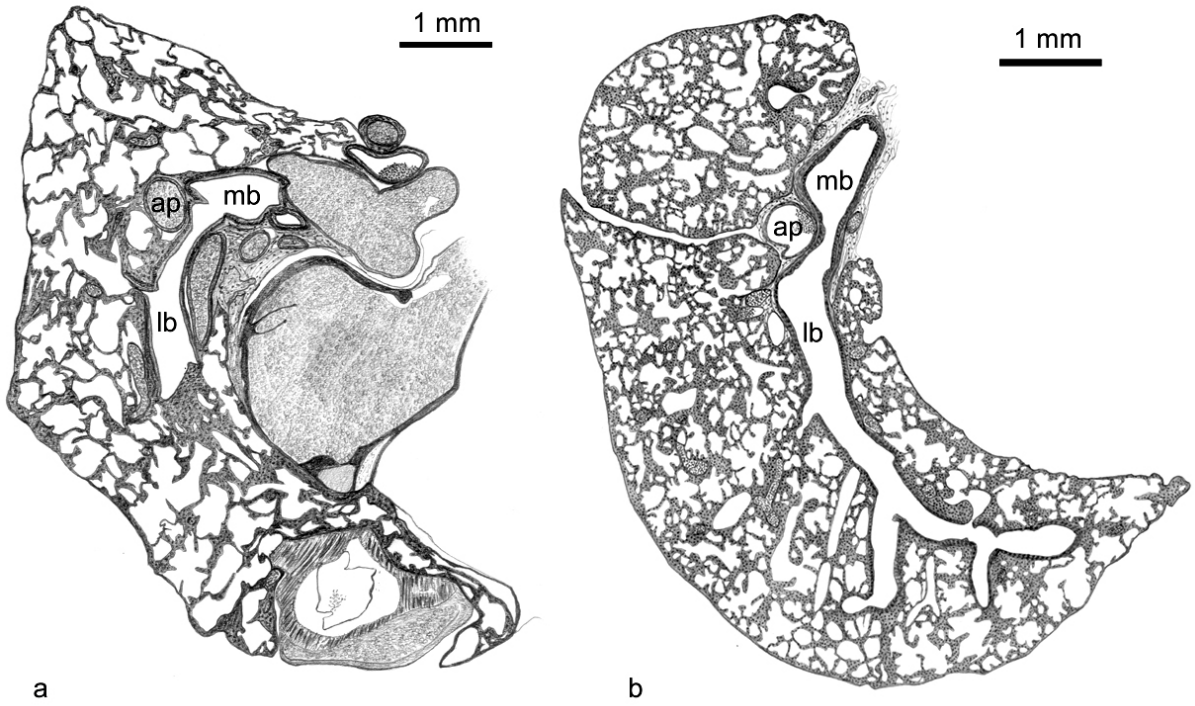

Fig. 90: Original drawings from histological sections of the right lung of a pouch young *Ornithorhynchus anatinus* (a) and a pouch young *Tachyglossus aculeatus* (b). ap, pulmonary artery; lb, lobar bronchus; mb, main bronchus. Magnification 25 x.

closed eyes and the claws of the fore and hind limbs are well developed. At this developmental stage no hairs or spines are present (fur appears at 30-40 days), but the skin is thick and the lungs appear to be already well developed (Fig. 89 b, 90 b). In comparison with the lung development of the marsupial *Monodelphis domestica* and in consideration of the developmental degree, the *Tachyglossus aculeatus* investigated may have an age of approximately 21 days. The pouch young of *Ornithorhynchus anatinus* was already sectioned. The histological sections through the anterior region of the pouch young *Ornithorhynchus anatinus* are more immature compared to the *Tachyglossus aculeatus.* The skin is relatively thin, the musculature is poor developed and the lungs are apparently less mature with large terminal air sacs (Fig. 89 a, 90 a). From these characters and in comparison with the marsupial lung, the age of the pouch young *Ornithorhynchus anatinus* was estimated to approximately seven days. A detailed description of the lung structure of both monotreme pouch young follows.

Ornithorhynchus anatinus

The lung of the pouch young *Ornithorhynchus anatinus* is at the early terminal sac stage of lung development. The bronchial tree is poorly developed and the gas exchange takes place

in the saccate terminal portions of the airways. The light microscopic findings of the lung of *Ornithorhynchus anatinus* are presented in the figure 91.

The lungs fill in nearly all blanks of the anterior region (Fig. 89 a, 91 a). They are variable in form to match with the other viscera. In the lungs of *Ornithorhynchus anatinus* no fissures are apparent, however, the branching pattern of the lobar bronchi indicates a subdivision in pulmonary lobes in both lungs. The right lung consists of superior, middle, accessory and inferior lobes and the left lung has superior, middle and inferior lobes.

The main bronchi measure 1 mm in diameter in the proximal parts and only a slight constriction of the lumen takes place in the more distal parts. The main bronchi are lined with a two layered cuboidal ciliated epithelium. Beneath a layer of three to four smooth muscle cells stabilises the bronchus. Cartilage supports the bronchial walls only extrapulmonar and ends when the main bronchus enters the lung. Loose connective tissue separates the main bronchi from the surrounding. From the main bronchi several lobar bronchi branch off. They measure 350-450 µm in diameter and the structure of the bronchial walls resembles that of the main bronchi. The bronchi, that branch off from the lobar bronchi, measure approximately 350 µm in diameter and are very short (Fig. 91 a). They give rise to smooth-walled channels, which measure 250 µm in diameter. They are lined with respiratory epithelium and end directly in the large terminal air sacs that are rich in capillaries (Fig. 91 b).

The terminal air sacs measure 250-350 µm in diameter. They are lined with respiratory epithelium and appear smooth-walled with only few septal crests protruding from the septa (Fig. 91 c). The thick septa separating the air sacs are septa with a double capillary network (Fig. 91 d). They have a thickness of 25-30 µm.

Tachyglossus aculeatus

The lung of the pouch young *Tachyglossus aculeatus* is likewise at the terminal sac stage of lung development. However, the lung is more advanced in the development. The light microscopic findings of the lung of *Tachyglossus aculeatus* are presented in the figure 91. The lungs of *Tachyglossus aculeatus* are conservative in their form (Fig. 89 b, 90 b). The right lung has completely separated superior, middle, accessory and inferior lobes. In the left lung no fissures are apparent, but the lobar bronchi indicate that the left lung is composed of fused middle and inferior lobes. The bronchial tree of *Tachyglossus aculeatus* is well developed and consists of main, lobar and segmental bronchi and bronchioles. But also smooth-walled channels are still present. The main bronchi measure 750 µm in diameter in the proximal parts. They are lined with one-layered cuboidal or columnar ciliated epithelium. Beneath a layer of two to three smooth muscle cells follows.

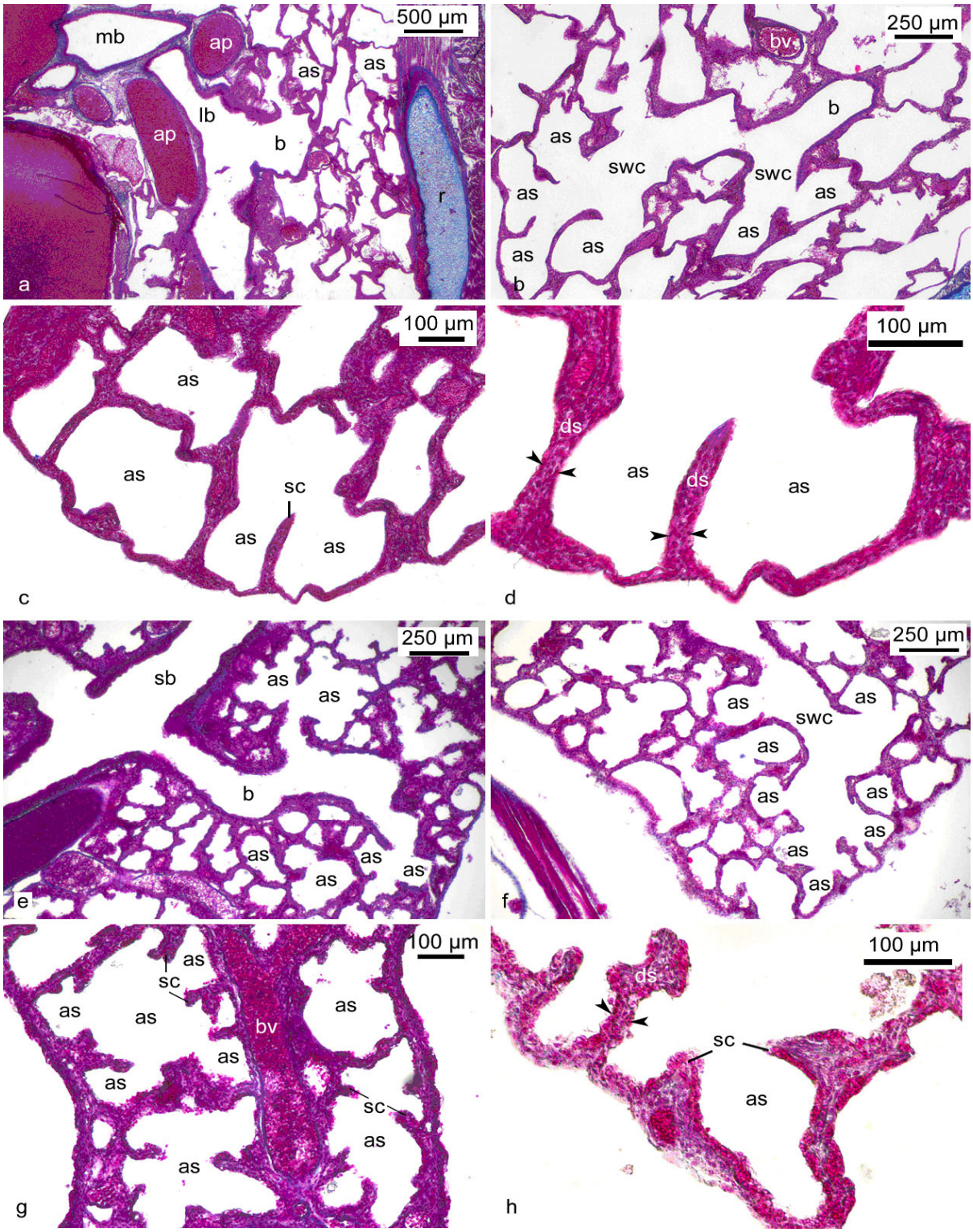

Fig. 91: Light micrographs of the lungs of a pouch young *Ornithorhynchus anatinus* (a-d) and a pouch young *Tachyglossus aculeatus* (e-h). Both lungs are at the terminal sac stage of lung development. The less developed bronchial tree (a, b) and the large smooth-walled terminal air sacs (c, d) of the *Ornithorynchus anatinus* indicate an early stage of lung development. In the *Tachyglossus aculeatus* the lung is more developed. The bronchial tree is ramified (e), but in the distal portions of the airways smooth-walled channels are still present (f). Numerous septal outgrowths subdivide the air sacs and give them an irregular shape (g, h). The air sacs are separated by septa with a double capillary network (arrowheads indicate capillaries). as, air sac; ap, pulmonary artery; b, bronchiole; bv, blood vessel; lb, lobar bronchus; mb, main bronchus; ds, double capillary septum; r, rib; sb, segmental bronchus; sc, septal crest; swc, smooth-walled channel. Azan staining. Magnification is indicated by the scale bar.

Cartilage supports the main bronchi only extrapulmonar and extends to the point, where the main bronchi enter the lungs. The lobar bronchi measure 300-350 µm. The composition of the bronchial walls corresponds to that of the main bronchi. From the lobar bronchi segmental bronchi with a diameter of 250-300 µm branch off and supply the peripheral areas of the lung. The terminal airways consist of bronchioles which give rise to smooth-walled channels (Fig. 91 e). The bronchioles have a diameter of 150-200 µm and are lined with simple cuboidal epithelium. The smooth-walled channels, which branch off from bronchioles, measure 100-150 µm in diameter and are lined with respiratory epithelium. They open into large terminal air sacs of 250-400 µm (Fig. 91 f). Numerous septal crests protrude from the septa into the air sac lumen and give them an irregular shape (Fig. 91 g). The septal outgrowths lead to a subdivision of the large air sacs into smaller air sacs of approximately 100-150 µm in diameter. The new septal crests as well as the older septa are septa with a double capillary bed (Fig. 91 h). They have a thickness of 20-30 µm.

3.2.5 Summary of lung development

All mammalian species investigated share some characters of lung structure. They all have four pulmonary lobes in the right lung, a superior lobe, a middle lobe, an accessory lobe and a inferior lobe. The bronchial and bronchiolar walls have a similar composition and they vary only in the thickness of the components. The ultrastructural investigations reveal similarities concerning the presence of squamous type I pneumocytes and cuboidal type II pneumocytes lining the air spaces. Lamellar bodies and surfactant are present in all lungs at birth. The blood-air barrier has a trilaminar structure in all species and varies only in thickness.

Besides these similarities, there are also some differences concerning the lung morphology, for example in the number of pulmonary lobes of the left lung. The comparative investigation of the lung development shows that the postnatal pulmonary maturation follows the same pattern in all mammalian species. However, the developmental degree of the neonatal lung and the speed of the postnatal pulmonary maturation differ remarkably between marsupial and placental mammals.

The findings of the lung development of the mammalian species examined are summarised in table 3.

Tab. 3: Data on lung development for the five placental and two marsupial species.

	Placentalia					Marsupialia	
Order	Rodentia	Eulipo-thyphla	Scan-dentia	Rodentia	Macro-scelidea	Didel-phoidea	Macro-podoidea
Species	*Meso-cricetus auratus*	*Suncus murinus*	*Tupaia belangeri*	*Cavia aperea*	*Macro-scelides proboscideus*	*Monodelphis domestica*	*Macropus eugenii*
Pulmonary lobes of left lung	middle, inferior lobes	inferior lobe	superior, middle, inferior lobes	middle, accessory, inferior lobes	superior, middle, inferior lobes	middle, inferior lobes	middle, inferior lobes
Development-al stage of lung at birth	air sac stage	air sac stage	alveolar stage	alveolar stage	alveolar stage	air sac stage	air sac stage
Size of air spaces at birth	50-70 µm	60-80 µm	15-25 µm	15-20 µm	10-15 µm	300-400 µm	150-300 µm
Septum at birth	Double capillary	Double capillary	Single capillary	Single capillary	Single capillary	Double capillary	Double capillary
Thickness of septa at birth	10-20 µm	10-15 µm	6 µm	7-9 µm	5 µm	30 µm	30-40 µm
Thickness of blood-air barrier at birth	300-400 nm	400-450 nm	200-250 nm	200-250 nm	n.i.	500-1000 nm	n.i.
Formation of alveoli	2 days	4 days	0 days	0 days	0 days	28 days	65 days
Respiratory bronchioles and alveolar sacs	7 days	7 days	0 days	0 days	0 days	28-41 days	142 days

n.i. not investigated

3.3 Metabolism during the early postnatal period

When comparing the metabolic rate of vertebrates the convention has been to use the concept of a standard metabolic rate (SMR). This is calculated from measurements of heat production or oxygen consumption in an organism under specified standard conditions. The conditions are usually that the organism is rested (or as near to rested as possible), fasting (if possible), awake, and in a thermoneutral environment (IUPS Thermal Commission, 2003). The SMR of adult animals is related to body weight (W) in an allometric manner, that is, SMR = a * W^b. Kleiber (1961) described this relationship in his equation SMR (ml O_2/g/h) = 3.42 $W(g)^{-0.25}$, which was for long time a standard for comparison among subsets of mammals and become known as the “mouse-to-elephant” curve. Several later studies, based on more extensive data sets, conclude that no single equation adequately describes the allometric relation between body mass and SMR for all mammals (Dawson and Hulbert, 1970; McNab, 1980, 1986; Hayssen and Lacy, 1985). Lee and Cockburn (1985) give two different formulae for the allometric relationship of SMR and body weight for marsupial and placental mammals respectively. Furthermore, Hayssen and Lacy (1985) suggest for Eutheria a line with a slope of -0.30 and for Metatheria a line with a slope of -0.25. According to these two studies, the following two formulae were used for the calculation of the respective expected mass specific SMR’s in this study:

- Marsupialia: SMR (ml O_2/g/h) = 2.33 $W(g)^{-0.25}$
- Placentalia: SMR (ml O_2/g/h) = 3.44 $W(g)^{-0.30}$

3.3.1 Marsupialia

Adult marsupials have a level of standard metabolism that is approximately 70 % of the rate for eutherian mammals (Dawson and Hulbert, 1970). The SMR is regarded as the minimum energy requirement necessary for maintenance of the living state of the particular animal thus measured (Hulbert, 1988). The measurement of the metabolic rate of the marsupial pouch young bears some difficulties. However, it is possible to offer subnatural conditions and to set similar criteria whereby the measured metabolic rate of pouch young can be compared both with each other and with adult rates.

3.3.1.1 Monodelphis domestica

Monodelphis domestica is a pouchless genus. The young are born in an embryonic condition, move actively to the mother´s teat and attach themselve firmly to the teat for the first 14 days of life. During this time, the young gain a certain degree of warmth from the

mother by the milk and body heath. But in contrast to marsupials with a pouch, the young *Monodelphis* are exposed to the environment when the mother is running (e.g. foraging). Then they have to cope with the ambient temperatures. To provide natural conditions as much as possible, the metabolic measurements of the young *Monodelphis domestica* were carried out together with the mother in thermal neutrality at ambient temperatures of 26-27.5°C. As a comparative value the respective females were already measured during the late pregnancy (corresponds to early lactation; see 2.3.3). In the postpartal measurements this value of the mother was subtracted and the difference was calculated for the litter size. From this calculation the value for an individual young of the litter resulted. This single values, resulting from the initial five (later four, than three) mother-young measurements were pooled for the respective age classes and presented as means with standard deviation. From the age of 21 days the young were measured separately at the same ambient temperatures. From this age the data from all measured young were pooled for the respective age classes and presented as means with standard deviation (Tab. 4). The mass specific standard metabolic rate and the standard metabolic rate of *Monodelphis domestica* are shown in the figures 92 and 93.

During the first 14 days of life the standard metabolic rate of *Monodelphis domestica* is extremely low and ranges from 0.09 ± 0.01 to 0.55 ± 0.25 ml O_2*h^{-1}. Between the age of 21

Tab. 4: Original data from the measurement of the oxygen consumption in *Monodelphis domestica*. The expected mass specific standard metabolic rate was calculated with Kleiber's equation: SMR= 2.33 W $(g)^{-0.25}$ (Lee and Cockburn, 1985; Hayssen and Lacey, 1985).

Age (day)	Number of animals	Mean ambient temperature (°C)	Mean body weight (g)	Standard metabolic rate (ml O_2*h^{-1}) Mean ± SD	Mass specific standard metabolic rate (O_2*g^{-1}*h^{-1}) Mean ± SD	Expected mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1})
0	5*	26.4	0.09	0.09 ± 0.01	0.95 ± 0.10	4.25
4	5*	26.5	0.21	0.20 ± 0.03	0.95 ± 0.16	3.46
7	4*	27.3	0.42	0.33 ± 0.03	0.79 ± 0.08	2.89
11	4*	27.0	0.64	0.47 ± 0.12	0.74 ± 0.18	2.61
14	3*	26.0	0.90	0.55 ± 0.25	0.61 ± 0.28	2.39
21	14	27.5	2.06	1.38 ± 0.68	0.61 ± 0.24	1.94
28	14	26.4	4.03	2.32 ± 0.74	0.56 ± 0.18	1.64
42	14	26.7	9.42	5.53 ± 3.60	0.66 ± 0.28	1.33
56	14	26.9	19.63	22.57 ± 4.95	1.19 ± 0.25	1.11
Adult	12	26.0	71.94	70.48 ± 16.52	0.98 ± 0.24	0.80

* number of litters measured; litter sizes 4, 5, 7, 8, 11 young

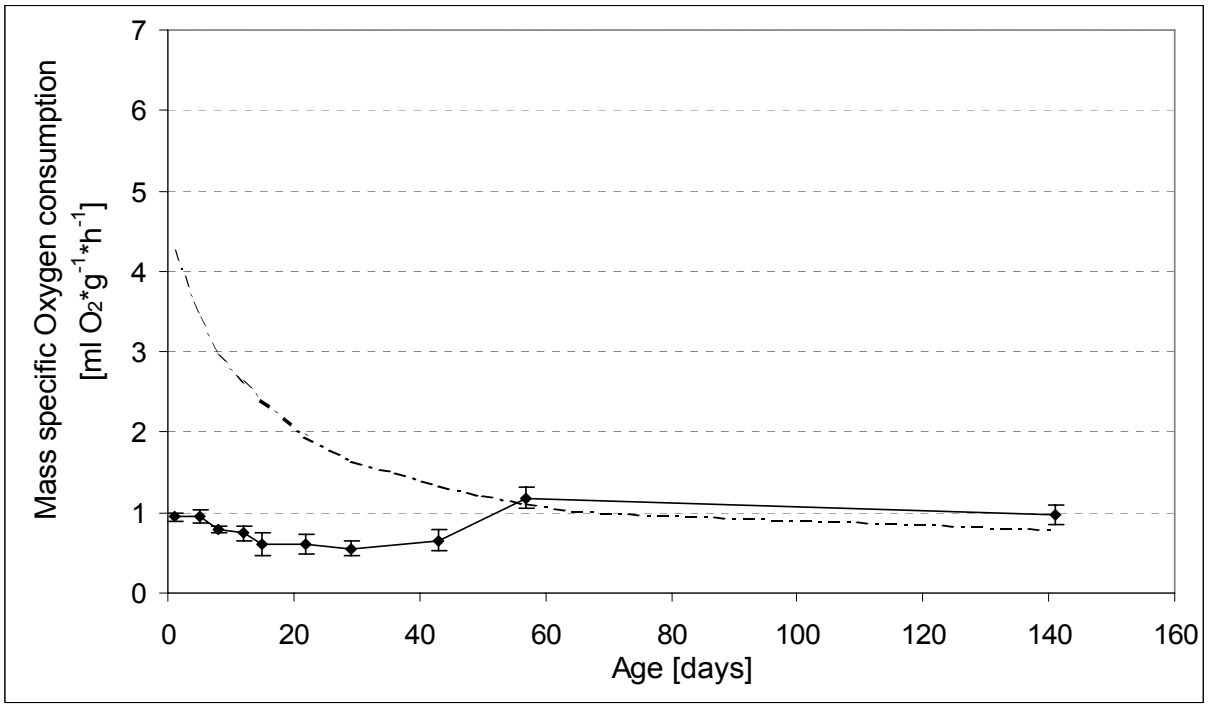

Fig. 92: Mass specific rates of oxygen consumption (VO_2) in *Monodelphis domestica* (solid line) as a function of age. The dashed line represents the level of VO_2 expected for marsupials (see table 4). Values presented as means with standard deviation of the respective age classes.

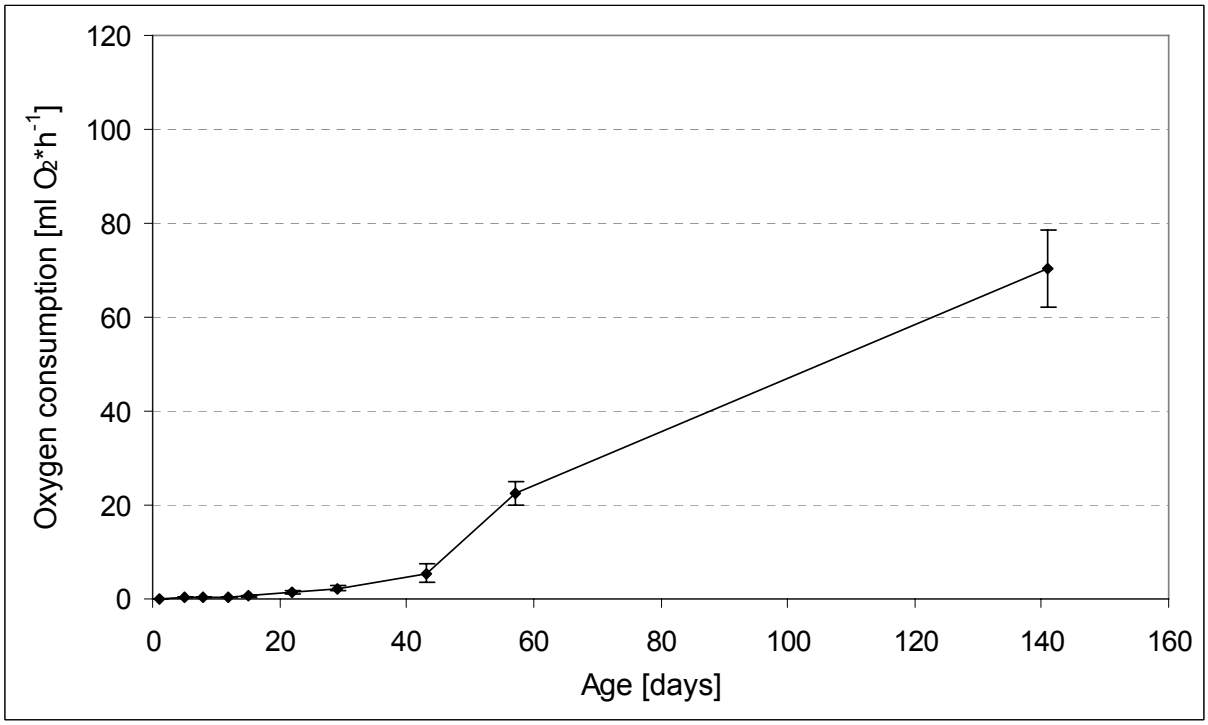

Fig. 93: Standard metabolic rate in *Monodelphis domestica* as a function of age. Values presented as means with standard deviation of the respective age classes.

and 42 days the SMR increases slowly from 1.38 ± 0.68 to 5.53 ± 3.60 ml O_2*h^{-1}. A sharp increase of the metabolic rate to 22.57 ± 4.95 ml O_2*h^{-1} takes place between 42 and 56 days. After that time the SMR rises to the adult level of 70.48 ± 16.52 ml O_2*h^{-1}. The delayed rise of the standard metabolic rate can be explained with the slowly postnatal growth rate in *Monodelphis domestica* (see chapter 3.1.2.1).

A better intra- and interspecific comparison of the metabolism offers the mass specific standard metabolic rate (VO_2) expressed in units of body weight. In consideration of the allometric relationship between metabolism and body weight, an expected mass specific standard metabolic rate can be calculated for *Monodelphis domestica,* using the Kleiber´s equation (Kleiber, 1961; corrected from Lee and Cockburn, 1985; Hayssen and Lacey, 1985). The expected mass specific standard metabolic rate is shown in table 3 and figure 92.

In the neonatal *Monodelphis domestica* the mass specific standard metabolic rate of 0.95 ± 0.10 ml O_2*g^{-1}*h^{-1} is very similar to the metabolic rates of adults (0.98 ± 0.24 ml O_2*g^{-1}*h^{-1}). Considered in total the oxygen consumption remains relatively unchangeably on a low level between 0.56 ± 0.18 and 0.95 ± 0.16 ml O_2*g^{-1}*h^{-1} within the first 6 weeks of life. Between 42 and 56 days the mass specific standard metabolic rate increases from 0.66 ± 0.28 to 1.19 ± 0.25 ml O_2*g^{-1}*h^{-1}. This rise, also noticeable in the standard metabolic rate, can be associated with the dietary change at the process of weaning, which occurs at this time. In comparison with the expected mass specific standard metabolic rate from the Kleiber´s equation, the actual mass specific standard metabolic rate of *Monodelphis domestica* remains below the expected values during the first eight weeks of life. In the neonate, the real mass specific standard metabolic rate of 0.95 ± 0.10 ml O_2*g^{-1}*h^{-1} equals the maternal level and, thus, amounts a fifth part or 22.4 % of the value to be expected from birth weight (4.25 ml O_2*g^{-1}*h^{-1}). *Monodelphis domestica* does not reach VO_2 levels predictable from body mass until 56 days.

3.3.1.2 Macropus eugenii

The newborn *Macropus eugenii* has a body mass of 370 mg; it is initially hairless and firmly attached to the maternal teat for 105 days. The pouch young spends altogether about 250 days dependent on the pouch. During this time the pouch young is accustomed to the thermoneutral environment of the pouch. For that reason the metabolic measurements of the pouch young *Macropus eugenii* were carried out at the constant ambient temperature of 37°C (similar to the temperature in the pouch). The rates of oxygen consumption are measured on pouch young aged between 5 and 140 days using indirect calorimetry. Due to the limited time during the research stay at the University of Melbourne, Australia only 22

single measurements were conducted. The data from all measured young are shown in table 5. The mass specific standard metabolic rate and the standard metabolic rate of *Macropus eugenii* are presented in the figures 94 and 95.

During the first 53 days of life the standard metabolic rate of *Macropus eugenii* is low and ranges from 0.47 to 5.20 ml O_2*h^{-1}. Between 53 and 65 days a noticeable size and weight gain of the pouch young takes place (from 15.55 g to 27.55 g). In connection with that also the SMR of *Macropus eugenii* rises from 3.73 to 23.40 ml O_2*h^{-1}. After 65 days the SMR rises steadily to the highest measured oxygen consumption (101.60 ml O_2*h^{-1}) at the age of 140

Tab. 5: Original data from the single measurements of the oxygen consumption in *Macropus eugenii* at an ambient temperature of 37°C. The expected mass specific standard metabolic rate was calculated with Kleiber's equation: SMR= 2.33 W $(g)^{-0.25}$ (Lee and Cockburn, 1985; Hayssen and Lacey, 1985).

Age (day)	Number of does	Sex	Head-length (mm)	Body weight (g)	Standard metabolic rate (ml O_2*h^{-1})	Mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1})	Expected mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1})
5	2404	m	9.7	0.87	0.47	0.54	2.41
10	1717	f	11.1	1.29	0.93	0.72	2.19
12	2362	m	11.9	1.41	0.93	0.67	2.14
19	2403	m	14.3	3.13	2.60	0.83	1.75
33	2082	m	18.3	5.58	3.74	0.67	1.52
39	2334	f	21.8	10.47	5.20	0.50	1.30
41	2356	m	22.9	10.55	5.20	0.49	1.29
47	2356	m	24.2	13.00	4.67	0.36	1.23
53	2356	m	26.7	15.55	3.73	0.24	1.17
65	2319	m	32.3	27.55	23.40	0.85	1.02
71	2319	m	32.2	32.60	17.67	0.54	0.98
77	2063	m	33.5	34.78	22.09	0.64	0.96
81	2311	m	38.9	48.90	13.35	0.27	0.88
87	2377	f	38.5	45.42	36.38	0.80	0.90
95	2377	f	39.9	52.95	30.92	0.58	0.86
104	2324	f	42.3	70.33	39.74	0.57	0.80
111	2324	f	44.4	74.11	70.70	0.95	0.79
118	2392	f	45.7	84.42	66.27	0.79	0.77
121	2352	m	47.1	82.66	79.52	0.96	0.77
123	2348	f	48.6	89.45	57.76	0.64	0.75
130	2348	f	49.0	91.94	97.14	1.06	0.75
140	2348	f	50.6	97.65	101.60	1.04	0.74

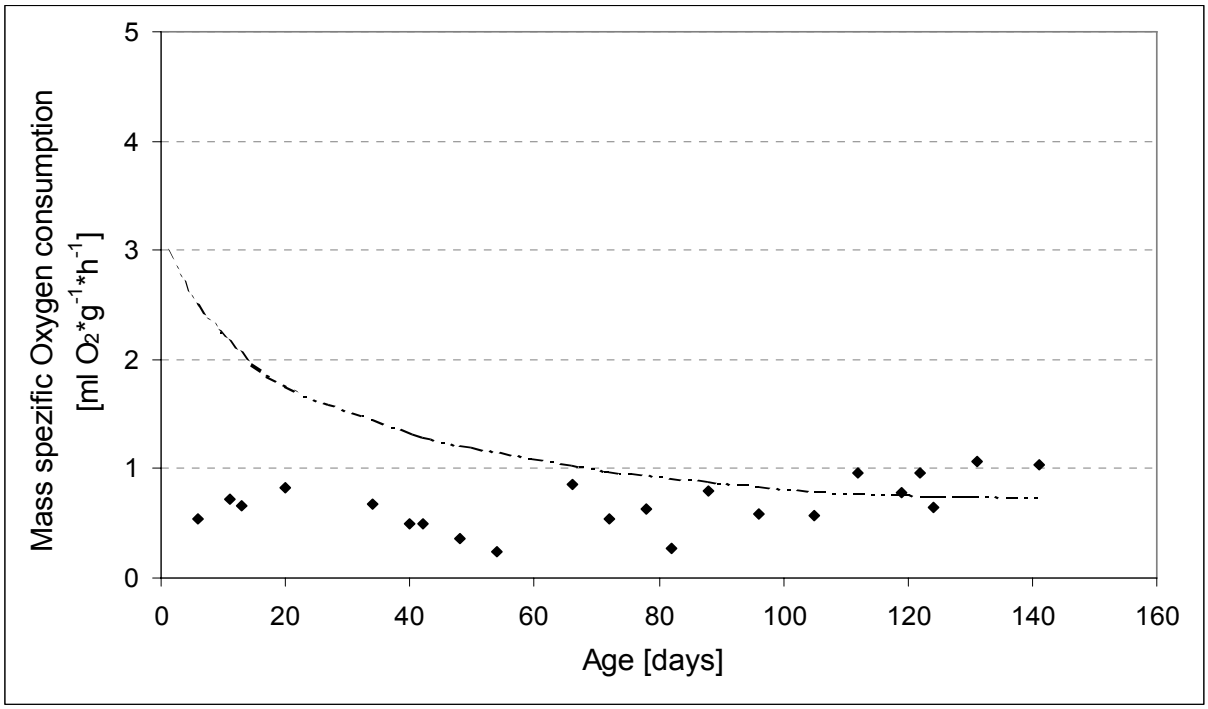

Fig. 94: Mass specific standard metabolic rate (VO_2) in *Macropus eugenii* (dots) as a function of age. The dashed line represents the level of VO_2 expected (see table 5) for marsupials.

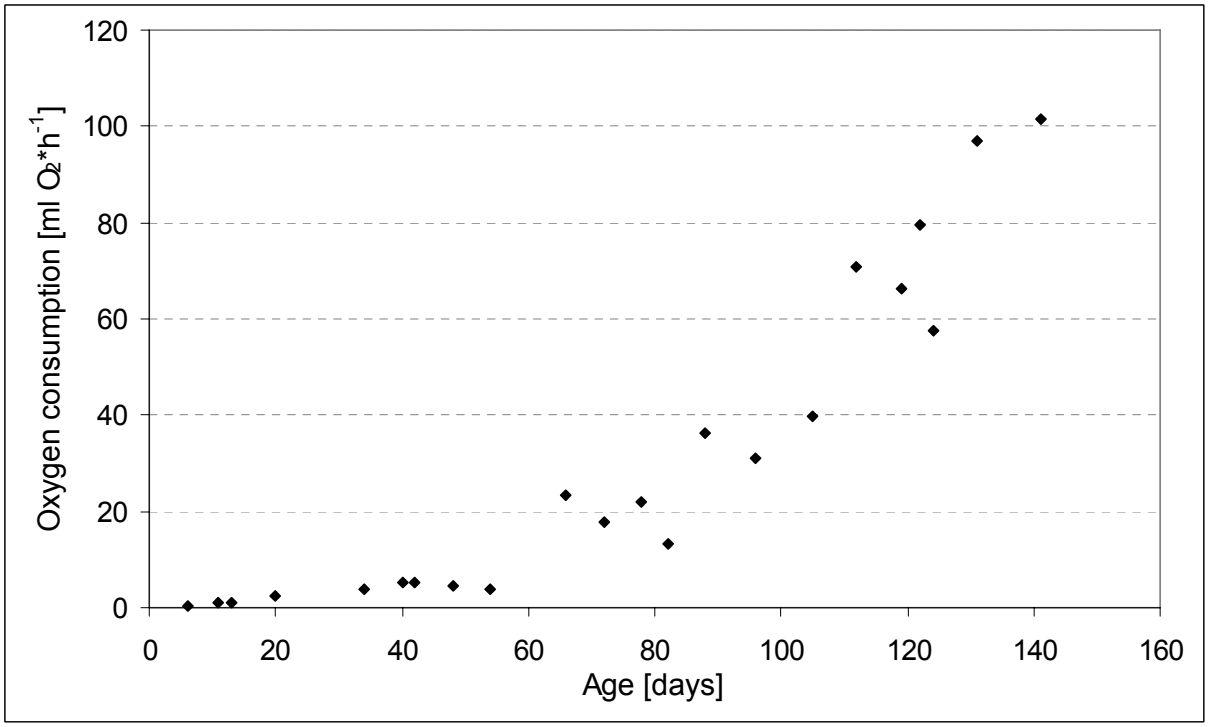

Fig. 95: Standard metabolic rate in *Macropus eugenii* as a function of age.

days. The curve of the standard metabolic rate (Fig. 95) bears resemblance to the growth curve of *Macropus eugenii* (see 3.1.2.2). As in *Monodelphis domestica,* a expected mass specific standard metabolic rate can be calculated also for *Macropus eugenii,* using the Kleiber´s equation (Kleiber, 1961; corrected from Lee and Cockburn, 1985; Hayssen and Lacey, 1985). The mass specific levels of oxygen consumption during the postnatal development are shown in table 5 and figure 94.

During a period from 5 to 140 days after birth no major changes in the mass specific standard metabolic rate become apparent. The mass specific standard metabolic rate remains on a low level between 0.24 and 1.06 ml $O_2*g^{-1}*h^{-1}$. In comparison with the expected mass specific standard metabolic rate from the Kleiber´s equation, the actual mass specific standard metabolic rate of *Macropus eugenii* remains below the expected values during the first 15 weeks of life. The mass specific standard metabolic rate of the five days old *Macropus eugenii* (0.54 ml $O_2*g^{-1}*h^{-1}$) amounts a fifth part or 22.4 % of the value to be expected from body weight (2.41 ml $O_2*g^{-1}*h^{-1}$). *Macropus eugenii* does not reach VO_2 levels predictable from body mass until 111 days.

3.3.2 Altricial Placentalia

Some species of small mammals are born in a relatively naked, uncoordinated, and helpless state and pass through a relatively long postnatal period of dependency on their parents. The altricial newborn, now dependent on its own resources, has to resist the hazards of survival imposed by hypothermia, desiccation and depletion of energy reserves. For that purpose a high neonatal metabolism, rapid metabolic development and an early onset of thermoregulation would be advantageous.

3.3.2.1 Mesocricetus auratus

Mesocricetus auratus is born in an altricial condition (hairless, closed eyes) with a birth weight of 2.1 g on the average. They have a slow growth rate during the first 14 days of life, but fur starts growing at the age of four days. The metabolic measurements were carried out at ambient temperatures in the thermoneutral zone of 27-28°C. The data from all measured young were pooled for the respective age classes and presented as means with standard deviation (Tab. 6). The mass specific standard metabolic rate and the standard metabolic rate of *Mesocricetus auratus* are shown in the figures 96 and 97.

The standard metabolic rate of the newborn *Mesocricetus auratus* is 3.53 $\pm$ 1.04 ml O_2*h^{-1}. During the first 11 days of life only a slow increase of the SMR to 8.65 $\pm$ 2.11 ml O_2*h^{-1} takes place. After that time the SMR rises steady to the adult level of 86.83 $\pm$ 14.00 ml O_2*h^{-1}. The

delayed rise of the metabolic rate matches with the slowly postnatal growth rate of *Mesocricetus auratus* during this time (see chapter 3.1.2.3). For an intra- and interspecific comparison of the metabolism, the mass specific standard metabolic rate (VO2) is expressed in units of body weight. From the Kleiber´s equation (Kleiber, 1961; corrected from Lee and Cockburn, 1985; Hayssen and Lacey, 1985) the expected mass specific standard metabolic rate of *Mesocricetus auratus* was calculated. The mass specific levels of oxygen consumption during the postnatal development are shown in table 6 and figure 96.

The mass specific oxygen consumption is with 1.81 ± 0.48 ml $O_2*g^{-1}*h^{-1}$ highest in the neonatal *Mesocricetus auratus.* The oxygen consumption expressed in units of body weight, is in the neonate twofold of that generally consumed by animals one week of age or older. During the consecutive days the mass specific standard metabolic rate decreases and stabilises at values of 0.76 ± 0.48 to 0.92 ± 0.14 ml $O_2*g^{-1}*h^{-1}$. This values are conform to the adult VO_2 of *Mesocricetus auratus* (0.76 ± 0.09 ml $O_2*g^{-1}*h^{-1}$). In comparison with the expected mass specific standard metabolic rate from the Kleiber´s equation, the actual mass specific standard metabolic rate of *Mesocricetus auratus* remains below the expected values during the first 21 days of life. However, the mass specific standard metabolic rate of the neonatal *Mesocricetus auratus* (1.81 ± 0.48 ml $O_2*g^{-1}*h^{-1}$) amounts 66 % of the value to be expected from body weight (2.75 ml $O_2*g^{-1}*h^{-1}$).

Tab. 6: Original data from the measurement of the oxygen consumption in *Mesocricetus auratus*. The expected mass specific standard metabolic rate was calculated with Kleiber's equation: SMR = 3.44 W $(g)^{-0.30}$ (Lee and Cockburn, 1985; Hayssen and Lacey, 1985).

Age (day)	Number of animals	Mean ambient temperature (°C)	Mean body weight (g)	Standard metabolic rate (ml O_2*h^{-1}) Mean ± SD	Mass specific standard metabolic rate (ml $O_2*g^{-1}*h^{-1}$) Mean ± SD	Expected mass specific standard metabolic rate (ml $O_2*g^{-1}*h^{-1}$)
0	12	27.4	2.10	3.53 ± 1.04	1.81 ± 0.48	2.75
4	12	27.6	5.03	3.84 ± 0.92	0.76 ± 0.16	2.12
7	12	27.7	7.08	5.95 ± 1.24	0.81 ± 0.15	1.91
11	9	27.4	11.61	8.65 ± 2.11	0.92 ± 0.14	1.65
14	9	27.7	15.93	14.39 ± 2.80	0.86 ± 0.12	1.50
21	9	27.8	37.22	30.01 ± 4.41	0.80 ± 0.12	1.16
Adult	8	27.0	95.47	86.83 ± 14.00	0.76 ± 0.09	0.88

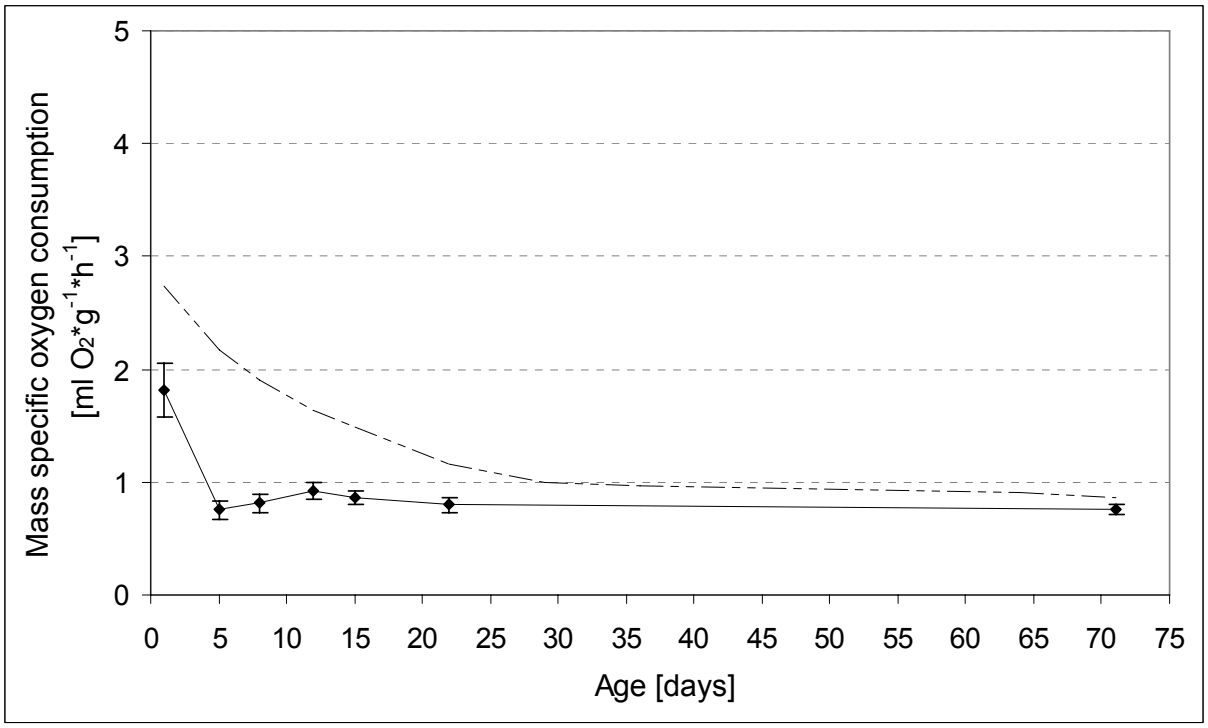

Fig. 96: Mass specific rates of oxygen consumption (VO_2) in *Mesocricetus auratus* (solid line) as a function of age. The dashed line represents the level of VO_2 expected (see table 6) for placentals. Values presented as means with standard deviation of the respective age classes.

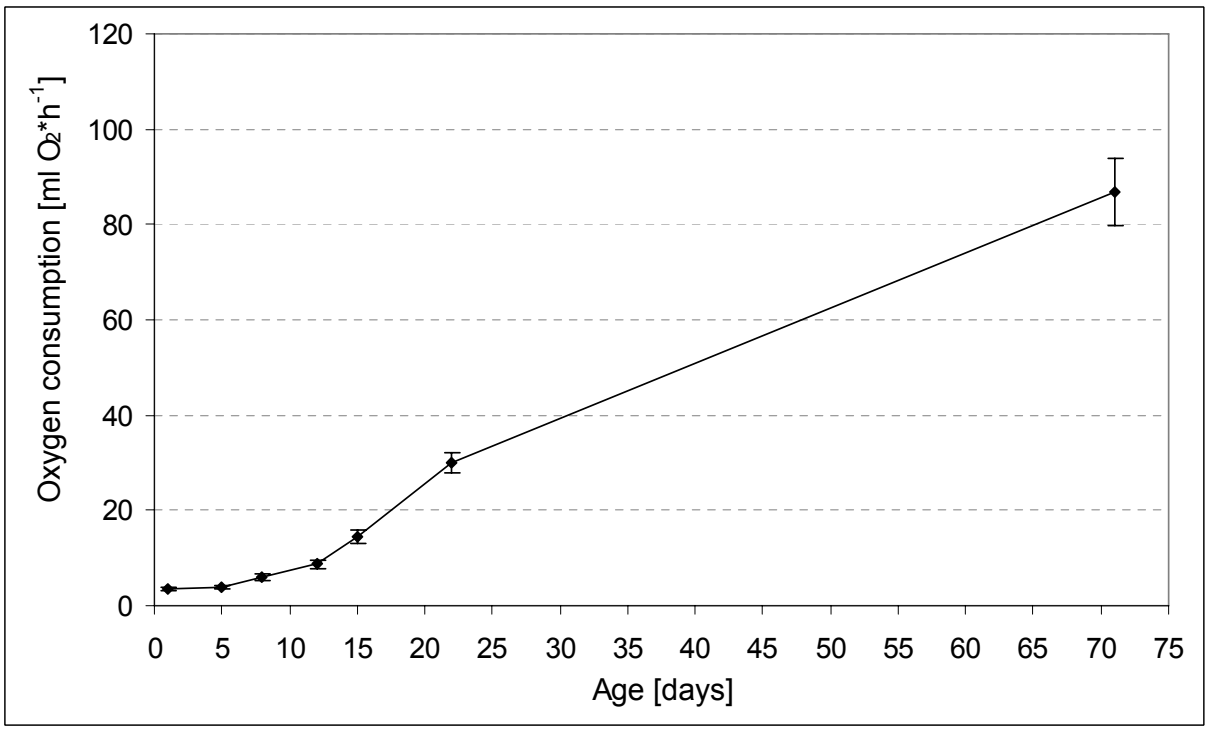

Fig. 97: Standard metabolic rate in *Mesocricetus auratus* as a function of age. Values presented as means with standard deviation of the respective age classes.

3.3.2.2 Suncus murinus

The newborn *Suncus murinus* is in an altricial condition (hairless, closed eyes) with a birth weight of 2.8 g on the average. It undergoes a rapid postnatal development with a high growth rate. Oxygen consumption in *Suncus murinus* was measured at 0, 4, 7, 11, 14 and 21 days of life and in adults. The metabolic measurements were carried out at ambient temperatures in the thermoneutral zone of 25-27°C. The data from all measured young were pooled for the respective age classes. The mass specific standard metabolic rate and the standard metabolic rate of *Suncus murinus* are presented in table 7 and in the figures 98 and 99. Compared to the newborn *Mesocricetus auratus,* the standard metabolic rate of the newborn *Suncus murinus* is with 12.34 ± 1.15 ml O_2*h^{-1} relatively high. During the first 21 days of life a steady increase of the SMR to 52.11 ± 6.45 ml O_2*h^{-1} takes place. With 21 days *Suncus murinus* has nearly reached the adult standard metabolic rate of 63.44 ± 11.62 ml O_2*h^{-1}. The course of the standard metabolic rate matches with the high postnatal growth rate of *Suncus murinus* (see chapter 3.1.2.4). Neonatal shrews consumed the most oxygen. The mass specific oxygen consumption in neonates amounts 4.43 ± 0.72 ml O_2*g^{-1}*h^{-1}. The oxygen consumption expressed in units of body weight, is in the neonate threefold of that generally consumed by animals one week of age or older. The actual neonatal mass specific standard metabolic rate is 43 % higher than the expected mass specific standard metabolic rate (2.53 ml O_2*g^{-1}*h^{-1}). These differences are not explained by the small size of the neonates since, after calculation of the expected mass specific standard metabolic rate from the Kleiber´s equation (Kleiber, 1961; corrected from Lee and Cockburn, 1985; Hayssen and

Tab. 7: Original data from the measurement of the oxygen consumption in *Suncus murinus*. The expected mass specific standard metabolic rate was calculated with Kleiber's equation: SMR = 3.44 W $(g)^{-0.30}$ (Lee and Cockburn, 1985; Hayssen and Lacey, 1985).

Age (day)	Number of animals	Mean ambient temperature (°C)	Mean body weight (g)	Standard metabolic rate (ml O_2*h^{-1}) Mean ± SD	Mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1}) Mean ± SD	Expected mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1})
0	9	27.3	2.79	12.34 ± 1.15	4.43 ± 0.72	2.53
4	9	27.6	7.78	20.80 ± 3.01	2.44 ± 0.57	1.86
7	9	27.7	13.24	27.64 ± 5.34	1.95 ± 0.54	1.58
11	9	27.3	20.80	35.68 ± 3.85	1.71 ± 0.31	1.38
14	9	27.7	24.61	37.49 ± 3.62	1.51 ± 0.30	1.32
21	9	27.7	36.69	52.11 ± 6.45	1.42 ± 0.35	1.17
Adult	8	27.6	60.80	63.44 ± 11.62	1.33 ± 0.41	1.01

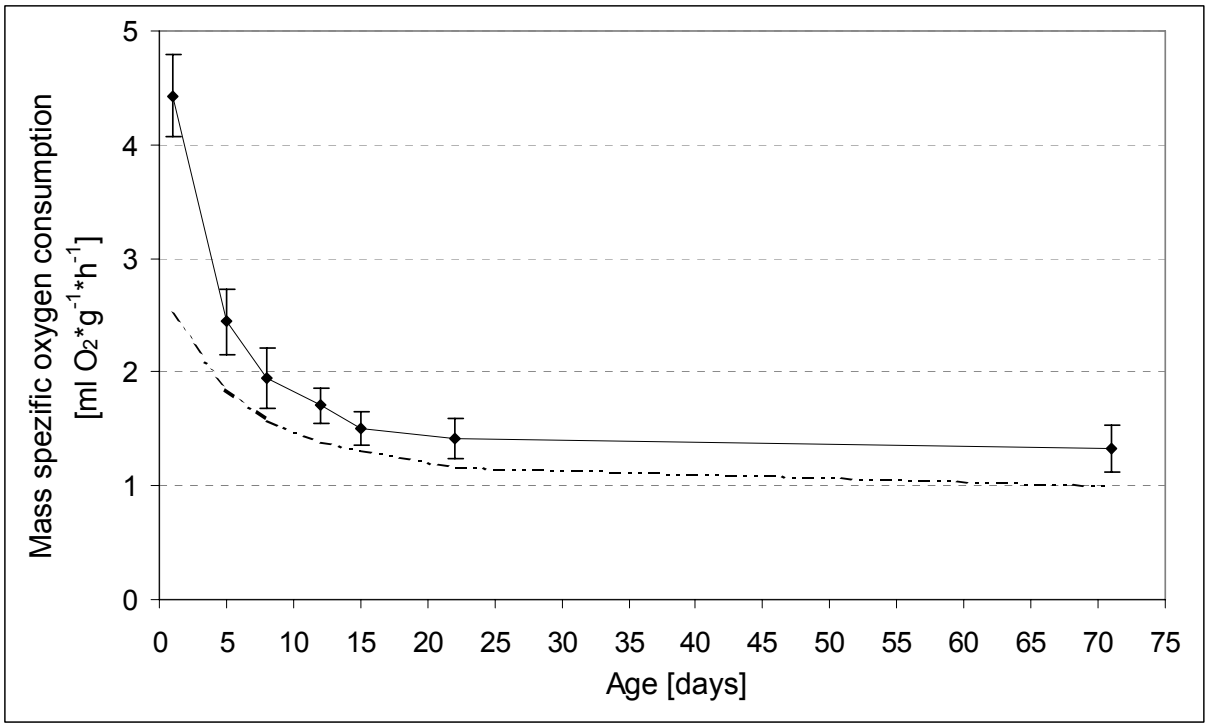

Fig. 98: Mass specific rates of oxygen consumption (VO_2) in *Suncus murinus* (solid line) as a function of age. The dashed line represents the level of VO_2 expected (see table 7) for placentals. Values presented as means with standard deviation of the respective age classes.

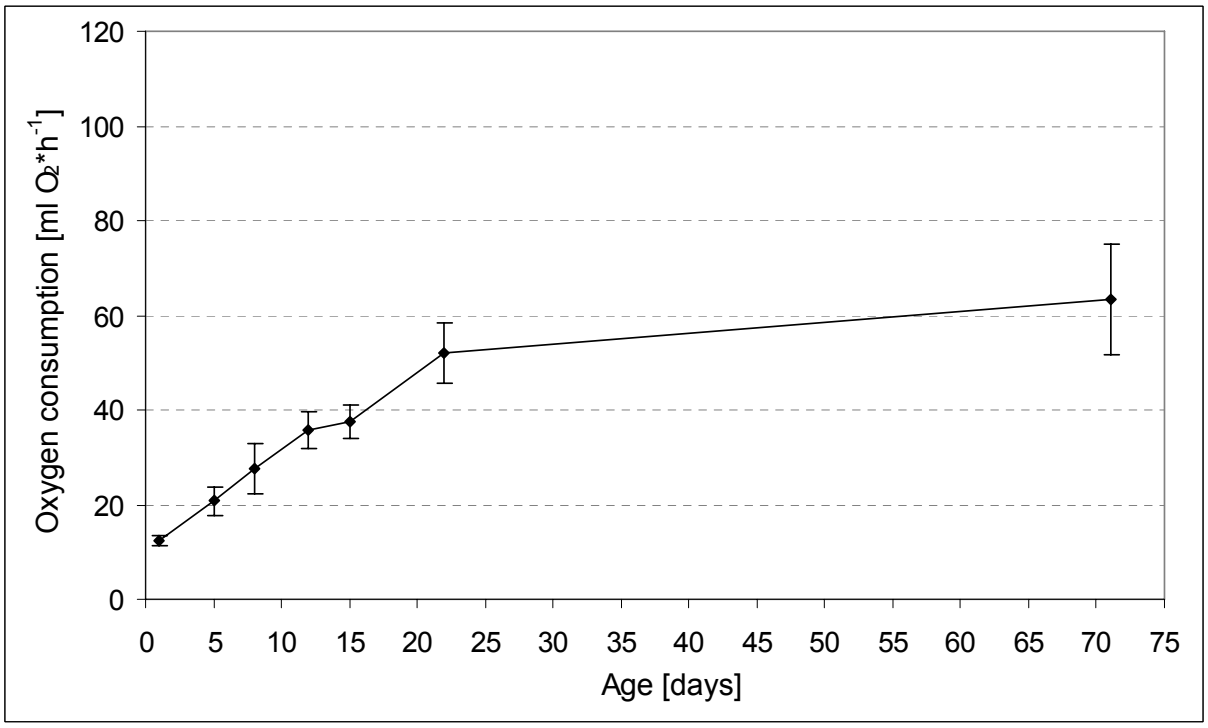

Fig. 99: Standard metabolic rate in *Suncus murinus* as a function of age. Values presented as means with standard deviation of the respective age classes.

Lacey, 1985), it is apparent that the greater metabolic rate is age-related. Oxygen requirements decrease during the consecutive days and stabilise at the age of seven days. From that age the mass specific standard metabolic rate is nearly conform to the expected mass specific standard metabolic rate.

3.3.2.3 Tupaia belangeri

The third altricial born Placentalia investigated is *Tupaia belangeri.* The young is born in an altricial condition with closed eyes and hairless, but with a relatively high birth weight of 18.3 g in average. The postnatal development of *Tupaia belangeri* is rapid. However, it is associated with a high degree of immobility. The metabolic measurements were carried out at ambient temperatures in the thermoneutral zone of young tupaias between 28 and 29 °C. The oxygen consumption was measured at 0, 4, 7, 11, 14 and 21 days of life. For comparison the adult metabolic rate of *Tupaia belangeri* was supplemented from Weigold (1979). The data from the nine measured young were pooled for the respective age classes. The original data are presented in table 8 and the curves of the mass specific standard metabolic rate and the standard metabolic rate are shown in the figures 100 and 101.

The newborn *Tupaia belangeri* has a standard metabolic rate of 39.93 ± 8.15 ml O_2*h^{-1}. During the first 14 days of life only a slow increase in the metabolic rate to 56.64 ± 12.63 ml O_2*h^{-1} takes place. From that time a marked increase of the metabolic rate to 96.67 ± 15.28 ml O_2*h^{-1} occurred. The mass specific oxygen consumption is highest in the neonatal

Tab. 8: Original data from the measurement of the oxygen consumption in *Tupaia belangeri*. The expected mass specific standard metabolic rate was calculated with Kleiber's equation: SMR = 3.44 W $(g)^{-0.30}$ (Lee and Cockburn, 1985; Hayssen and Lacey, 1985).

Age (day)	Number of animals	Mean ambient temperature (°C)	Mean body weight (g)	Standard metabolic rate (ml O_2*h^{-1}) Mean ± SD	Mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1}) Mean ± SD	Expected mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1})
0	9	28.4	18.30	39.93 ± 8.15	2.22 ± 0.45	1.44
4	9	28.4	30.19	43.26 ± 11.67	1.45 ± 0.39	1.24
7	9	28.8	32.52	45.66 ± 9.35	1.12 ± 0.24	1.21
11	9	28.5	47.77	50.50 ± 8.69	0.99 ± 0.12	1.08
14	9	28.2	67.86	56.64 ± 12.63	1.02 ± 0.11	0.97
21	9	28.1	86.38	96.67 ± 15.28	1.00 ± 0.13	0.90
Adult	-	-	186*	154.38*	0.83*	0.72

* Values of adult *Tupaia belangeri* from Weigold (1979)

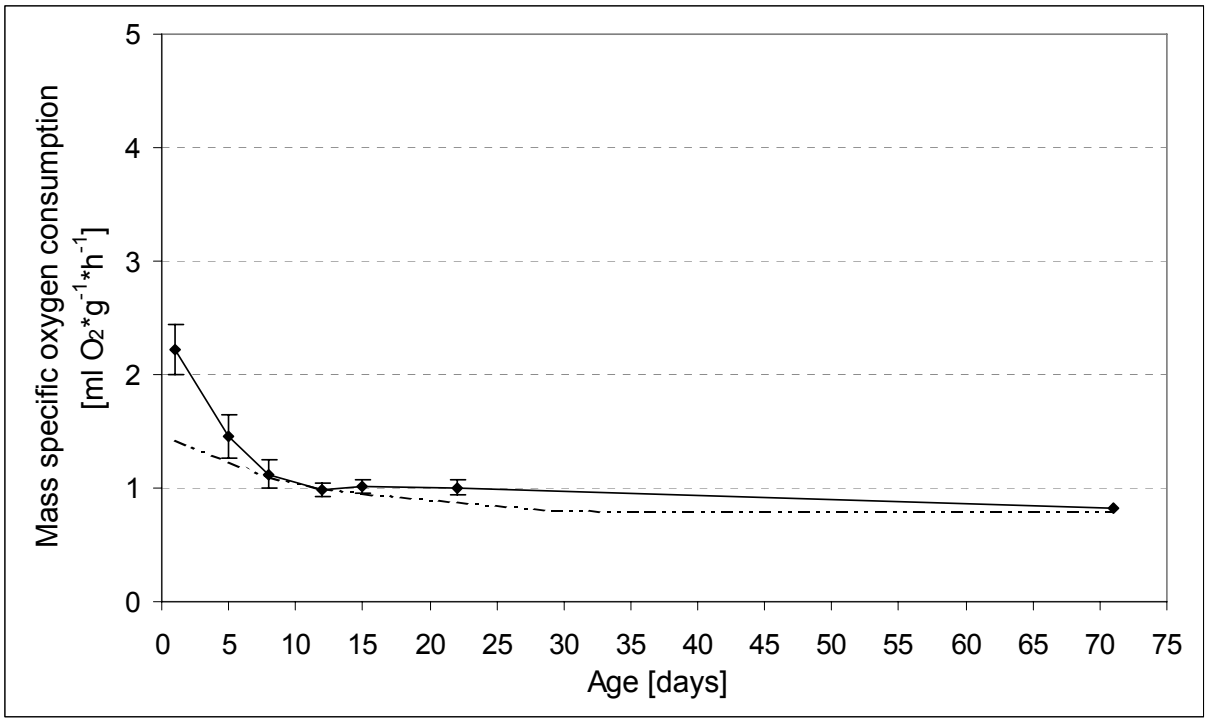

Fig. 100: Mass specific rates of oxygen consumption (VO_2) in *Tupaia belangeri* (solid line) as a function of age. The dashed line represents the level of VO_2 expected (see table 8) for placentals. Values presented as means with standard deviation of the respective age classes.

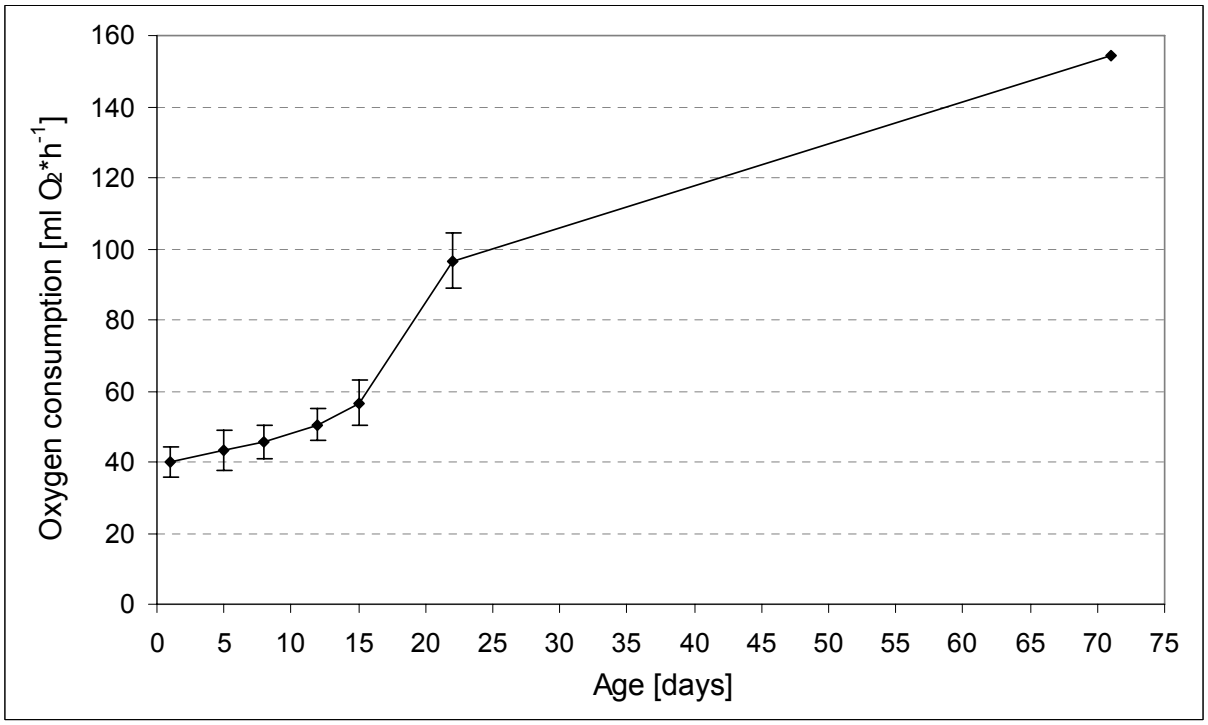

Fig. 101: Standard metabolic rate in *Tupaia belangeri* as a function of age. Values presented as means with standard deviation of the respective age classes.

Tupaia belangeri and amounts 2.22 ± 0.45 ml O_2*g^{-1}*h^{-1}. During the consecutive days the VO_2 decreases and stabilises from day 7 at values of 0.99 ± 0.12 to 1.12 ± 0.24 ml O_2*g^{-1}*h^{-1}. This values are nearly conform to the adult VO_2 of *Tupaia belangeri* (0.83 ml O_2*g^{-1}*h^{-1}) measured by Weigold (1979). In comparison with the expected VO_2 from the Kleiber´s equation, the actual neonatal mass specific standard metabolic rate is 35 % higher than the expected mass specific standard metabolic rate (1.44 ml O_2*g^{-1}*h^{-1}). From the age of seven days, the VO_2 of *Tupaia belangeri* conforms to the expected VO_2.

3.3.3 Precocial Placentalia

Among the Placentalia, some species are more developmentally advanced at birth. They are typically furred at birth or soon after; their eyes typically open soon; and they mature comparatively rapidly in locomotor ability and other respects, achieving independence of their parents relatively early. Beside the benefits of that precocial development, for example avoidance of predators and surviving the death of the parents, postnatal development of precocial mammals is energetically costly (Hackländer et al., 2002). A high metabolism and thermoregulatory abilities are necessary for that independent way of life.

3.3.3.1 Cavia aperea

As a representative for precocial born placentalia *Cavia aperea* was investigated. The young of *Cavia aperea* is born in a highly precocial condition. It has open eyes, it is completely furred and it is able to coordinate locomotion. The metabolic measurements were carried out at ambient temperatures in the thermoneutral zone of *Cavia aperea* between 24 and 25 °C. The oxygen consumption of *Cavia aperea* during the postnatal development was measured at 0, 4, 7, 11, 14 and 21 days of life. For comparison the adult metabolic rate of *Cavia porcellus* (domestic form of *C. aperea*) was supplemented from Künkele and Trillmich (1997). The original data are presented in table 9 and the curves of the mass specific standard metabolic rate and the standard metabolic rate are shown in the figures 102 and 103. The neonatal *Cavia aperea* has the highest standard metabolic rate of all six species investigated. It amounts 115.93 ± 13.83 ml O_2*h^{-1}. From four days after birth the SMR increases steady to 186.54 ± 4.84 ml O_2*h^{-1}at the age of 21 days. Since the growth of *Cavia aperea* continues after that time, also a further increase of the SMR can be assumed until the adult metabolic rate is attained. The course of the standard metabolic rate matches with the postnatal growth rate of *Cavia aperea* (see chapter 3.1.2.6). The mass specific oxygen consumption (VO_2) is with 1.82 ± 0.28 ml O_2*g^{-1}*h^{-1} highest in the neonat *Cavia aperea.* During the postnatal development a slight decrease the mass specific oxygen consumption

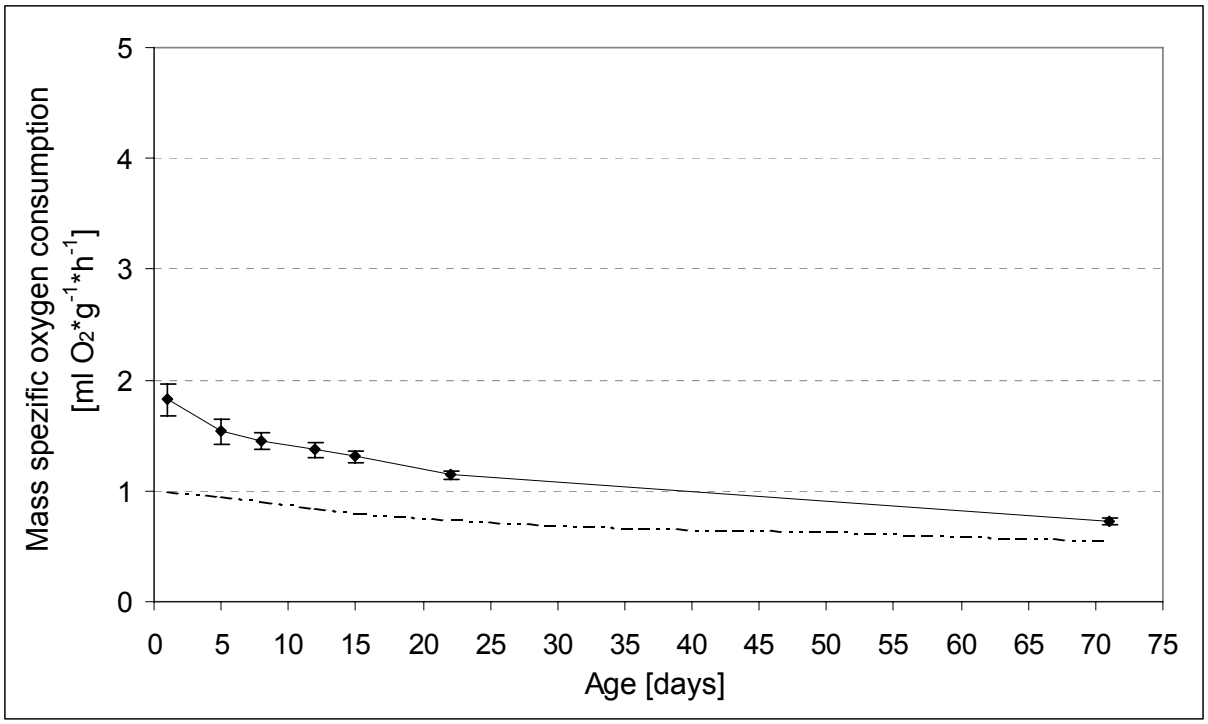

Fig. 102: Mass specific rates of oxygen consumption (VO_2) in *Cavia aperea* (solid line) as a function of age. The dashed line represents the level of VO_2 expected (see table 9) for placentals. Values presented as means with standard deviation of the respective age classes.

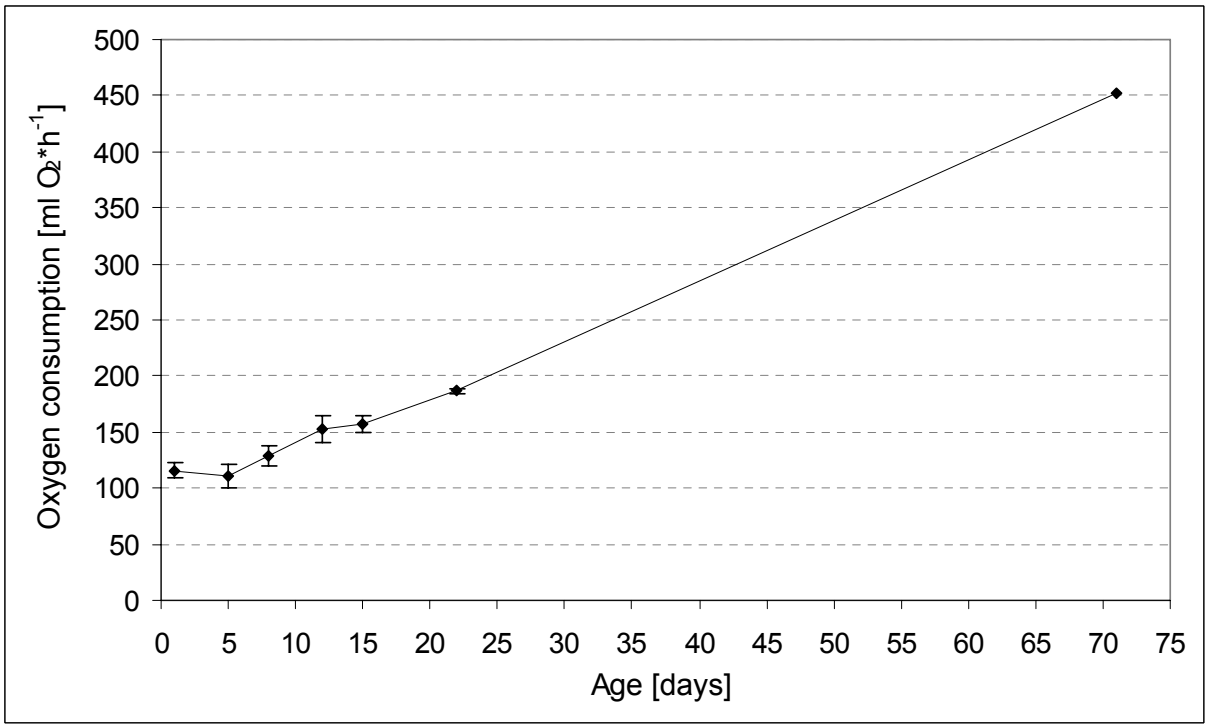

Fig. 103: Standard metabolic rate in *Cavia aperea* as a function of age. Values presented as means with standard deviation of the respective age classes.

Tab. 9: Original data from the measurement of the oxygen consumption in *Cavia aperea*. The expected mass specific standard metabolic rate was calculated with Kleiber's equation: SMR = 3.44 W $(g)^{-0.30}$ (Lee and Cockburn, 1985; Hayssen and Lacey, 1985).

Age (day)	Number of animals	Mean ambient temperature (°C)	Mean body weight (g)	Standard metabolic rate (ml O_2*h^{-1}) Mean ± SD	Mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1}) Mean ± SD	Expected mass specific standard metabolic rate (ml O_2*g^{-1}*h^{-1})
0	12	24.8	60.51	115.93 ± 13.83	1.82 ± 0.28	1.00
4	12	24.8	70.78	110.52 ± 20.57	1.54 ± 0.23	0.96
7	12	24.2	86.93	128.93 ± 17.27	1.45 ± 0.14	0.90
11	11	24.6	107.91	152.90 ± 24.96	1.37 ± 0.14	0.84
14	11	24.3	125.92	157.36 ± 13.86	1.31 ± 0.10	0.81
21	11	24.0	163.56	186.54 ± 4.84	1.15 ± 0.07	0.75
Adult	-	-	624.30*	451.99*	0.72 ± 0.07*	0.41

* Values of adult *Cavia porcellus* from Künkele and Trillmich (1997).

takes place. In comparison with to 1.15 ± 0.07 ml O_2*g^{-1}*h^{-1} at the age of 21 days

the expected VO_2 from the Kleiber´s equation, the actual mass specific standard metabolic rate runs above the expected mass specific standard metabolic rate. That can trace back to different reasons. On the one hand, the expected mass specific standard metabolic rate from the Kleiber´s equation may inadequately re-present the real metabolic rate of *Cavia aperea.* Or on the other hand, the measured VO_2 are not solely resting metabolic values, because the precocial young of *Cavia aperea* were more excited during the measurements than the altricial species, which generally slept. However, from the age of four days, the curve progression of the VO_2 in *Cavia aperea* is conform to that of the expected VO_2, merely on a higher level.

3.3.4 Summary of metabolic development

Summarising the metabolic development of the six mammalian species examined, the great differences between Placentalia and Marsupialia become clear. For an intra- and interspecific comparison of the metabolism, the mass specific standard metabolic rate (VO_2) expressed in units of body weight can be used. The mass specific standard metabolic rates of the four placental mammals are compared in figure 104 and the mass specific standard metabolic rates of the two marsupial mammals are summarised in figure 105. The most placental mammals are born with high mass specific standard metabolic rates. The VO_2 of placental newborns is usually higher than that in the adults of the same species, and also larger than that expected from their body weight (With the exception of *Mesocricetus*

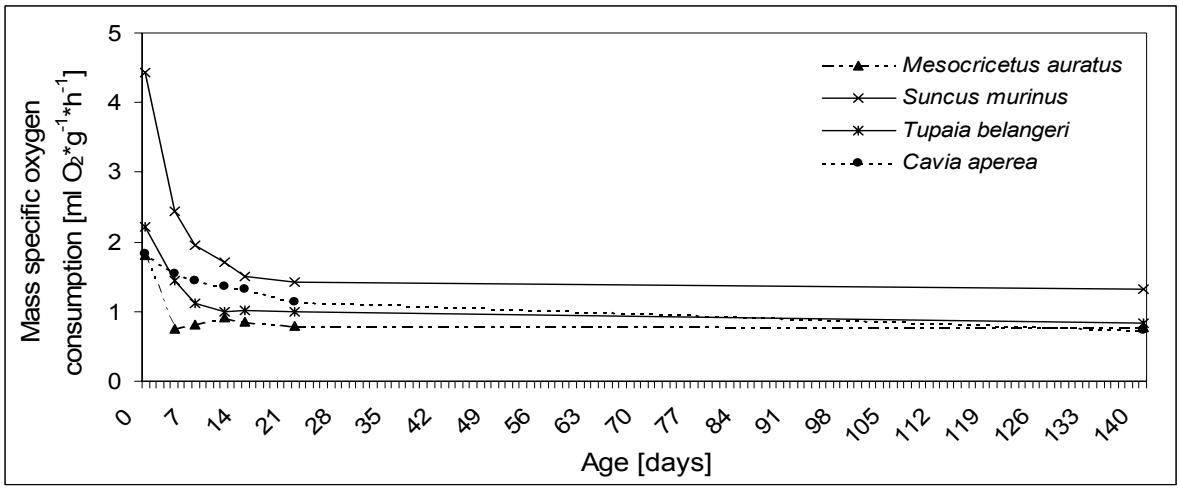

Fig. 104: Mass specific rates of oxygen consumption (VO_2) in the altricial Placentalia *Mesocricetus auratus, Suncus murinus* and *Tupaia belangeri* and in the precocial Placentalia *Cavia aperea* during the postnatal development.

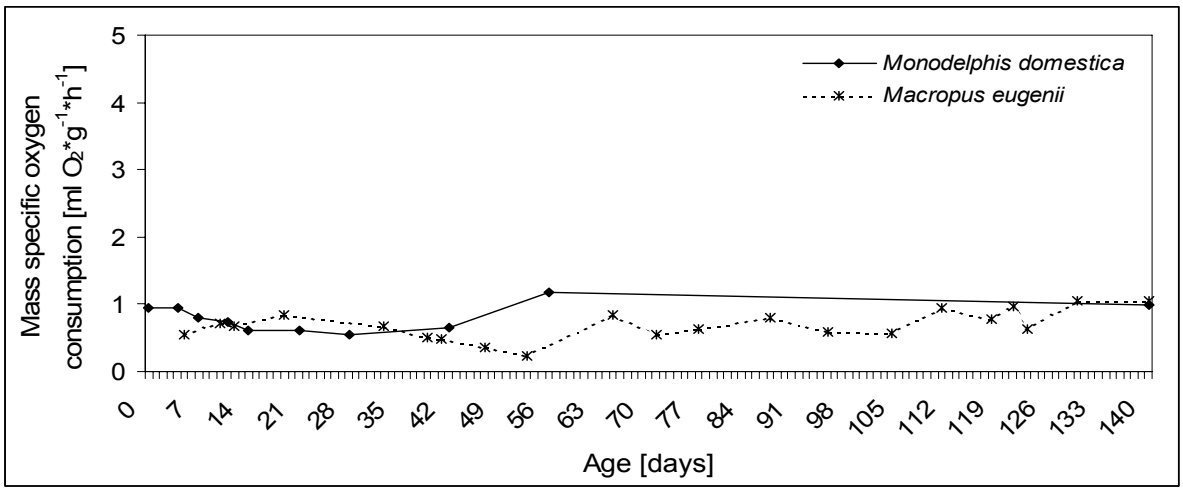

Fig. 105: Mass specific rates of oxygen consumption (VO_2) in the Marsupialia *Monodelphis domestica* and *Macropus eugenii* during the postnatal development.

auratus). After the first week of life, the young reach the adult metabolic rate.

In contrast to that, the marsupial neonates have a low VO_2 and maintain the low metabolic rate for a long time. They do not reach VO_2 levels expected from body mass until 56 days in *Monodelphis domestica* and 111 days in *Macropus eugenii*.

4 Discussion

4.1 General characteristics of neonates and postnatal development

Comparing the neonates of marsupials and placentals, some striking differences become apparent. Neonatal marsupial young are extremely small, with body weights 4 to 5 orders of magnitude less than the mother, for example a young Tasmanian devil weighs about 30 mg compared to the maternal weight of about 10 kg (Janssens et al., 1997). The two marsupials investigated fit into this pattern. *Monodelphis domestica* weighs at birth 100 mg, but as adult 80-100 g and *Macropus eugenii* has a birth weight of 370 mg and weighs 5 kg as adult. In contrast the body weights of most placental young are only 1 to 2 orders of magnitude less than the mother. That is the case in *Mesocricetus auratus* (neonate: 2.1 g; adult 90-110 g), *Suncus murinus* (neonate: 2.8; adult: 38-46 g), *Tupaia belangeri* (neonate: 18.3 g; adult: 151-212 g) and *Cavia aperea* (neonate: 60.5 g; adult: 400-600 g). Even the smallest placental neonate in relative terms, the giant panda, weighs about 100 g at birth compared to a maternal weight of about 100 kg, only 3 orders of magnitude difference (Ramsay and Dunbrack, 1986). The variety of birth weights of marsupial and placental mammals are presented in table III of the appendix.

4.1.1 Marsupialia

Despite the small size, marsupial neonates also appear undeveloped when compared to placentals and many organ systems are relatively poorly developed at birth (Janssens et al, 1997). However, the neonatal marsupial organ systems have to be mature enough to breathe air, suck milk, crawl in a directional manner and respond to sensory inputs. Hughes and Hall (1988) arrange the newborn marsupials into three grades of developmental complexity of their organ systems. The grades are manifested on the cytological level as well as on the external form of newborn. The least advanced is *Sarcophilus harrisii* (developmental stage G1) and the most advanced are *Macropus eugenii* and *M. giganteus* (developmental stage G3) with *Isoodon macrourus* (developmental stage G2) being intermediate (Hughes and Hall, 1988). The developmental stage G1 includes the lack of a definitive neck, a pronounced caudal taper, pronounced cervical swelling between the forearms, undifferentiated hindlimb paddles, recurved claws, an extensive oral shield, barely visible eye and ear primordia and prominent nasal swellings. Typical for the developmental stage G2 are pronounced forelimbs with claws, undifferentiated hindlimb paddles, a simple oral shield, visible eye and ear primordia and moderate nasal swellings. G3 is characterised by a definitive neck, advanced forelimbs, prominent eye and ear primordia, visible eyelides, a

vestigial oral shield and small nasal swellings. Among the marsupials, the newborns of the Dasyuroidea are at the developmental stage G1, the newborns of the Didelphoidea and Perameloidea are at the developmental stage G2 and the newborns of Diprotodontia are at the developmental stage G3. In this grading system *Monodelphis domestica* is equal to G2 and *Macropus eugenii* is equal to G3.

Sharman (1973) describes four distinct phases during the development of marsupial young:

1. A period of intrauterine development varying, in different species, from 12 to 38 days.
2. A period of free existence after birth, of several minutes duration only, during which the newborn young reaches the pouch by independent effort.
3. An extended period of development in the pouch varying, in different species, from about 2 to 11 months.
4. A period of independence of the pouch during which milk feeding continues. This is equivalent to the suckling period of placentals such as horse and ruminants, which are born in an advanced state of development.

Especially during phase 2, after birth and before pouch occupation the newborn marsupial is very vulnerable. The small size of the marsupial neonate predisposes it to hypothermia and desiccation. Therefore it needs adaptations that optimise the transfer to the pouch (Hughes and Hall, 1988). This is manifested externally by the well-developed and precociously pronated forelimbs, which have the capacity for digito-palmar prehension, and hypertrophy of the locomotory muscles of the torso. All marsupials are born with claws on their forelimbs which are used to grasp the mother's fur as the young climbs to the pouch, and to grip the fur around the teats after attachment (Sharman, 1973; Russell, 1982). The well-developed muscluar system of the brachial plexus, as described in the newborn *M. domestica*, is a prerequisite for the climbing in the fur. The claws are controlled by functional flexor and extensor muscles (Sharman, 1973). The hindlimbs by contrast are unrotated paddles which project at right-angles to the main axis of the body and are very much retarded in their developmental profiles when compared to the forelimbs (Hughes and Hall, 1988). That refers to the fact that the hindlimbs play no active role in the crawling movement of the newborn marsupial. Besides the movement to the maternal teat, also a firm attachment to the teat is important in newborn marsupials. The buccal cavity and pharynx of the newborn marsupials show structural adaptations that permit attachment to the teat, and allow breathing and feeding to proceed simultaneously (Sharman, 1973). The tongue is large with its intrinsic and chief extrinsic muscles fully established, with large hypoglossal nerves innervating them. When the teat is drawn into the buccal cavity the tongue assumes a semi-tubular form, and

the ridged hard palate becomes intended so that a tubular structure of pouch young tissue surrounds the teat. A bulbous swelling on the end of the teat, within the buccal cavity, is formed during the first few days of pouch life (Merchant and Sharman, 1966). That leads to a firm attachment of the young. In particular in the pouchless *Monodelphis domestica* a firm attachement of the young to the maternal teat is necessary to avoid the loss of the young. For this reason an oral shield is closely apposed to the skin around the base of each teat. Hill and Hill (1955) suggested that the oral shield acts as a "more or less air-tight washer", when the mouth is applied to the teat by coming into contact with the surrounding skin. A similar structure is found in the newborn native cat (Hill and Hill, 1955) and in the newborn Virginian opossum, but is apparently absent in neonatal bandicoots, brush possums, koalas and kangaroos which are more advanced at birth (Sharman, 1973).

The optic and auditory organs of the newborn marsupial are in an embryonic condition, but in all newborn marsupials the nostrils are open and large, and the olfactory organs appear capable of functioning (Sharman, 1973; Hughes and Hall, 1988). There is no evidence that the female marsupial assists the passage of the young from urogenital opening to teat other than by adopting a position that does not hinder the progress of the young by its own efforts (Sharman and Calaby, 1964; Russell, 1982; Renfree et al., 1989; Gemmell et al., 2002). However, the presence of large nostrils and of an apparent sense of smell suggests that newborn marsupials may be guided to the pouch by olfactory stimuli.

The skin of both marsupial neonates investigated is thin, semi-transparent, pinkish in colour and with a conspicuous network of blood vessels. Paris et al. (2005) provided a detailed description of the changes in the appearance of pouch young after birth. Initially the skin was fiery red with prominent blood vessels (until 7 hours post partum), than they became dull-red-cloured (between 6 and 19 hours post partum) and finally turned pink (from 17.6 hours post partum). The neonatal skin provides no isolation and is vulnerable. Due to the lesser thickness and the high vascularisation of the skin, a cutaneous respiration was suggested by Sharman (1973). In *Macropus eugenii*, skin vascularity is sparse with diffusion distances to the air of greater than 100 µm. In contrast, pulmonary vascularity is rich and with diffusion distances less than 1 µm (Randall et al., 1984). Baudinette et al. (1988b) reported for pouch young *M. eugenii* that only 2 % of the oxygen uptake occurs transcutaneously, however, more recent studies found cutaneous respiration in the newborn *Macropus eugenii* with a part of 30 % at the entire respiration (MacFarlane and Frappell, 2001). But it seems to be more important in very small marsupial neonates, such as *Sminthopsis douglasi*, were the skin contributes almost the total gaseous metabolism (Mortola et al., 1999; Frappell and Mortola, 2000).

The external morphology of the newborn marsupial is obviously influenced by the development of internal structures. In newborn of varying size, tissue maintenance

presumably varies in such a way that the larger newborns require a disproportionate increase in those organs where surface area is related to tissue maintenance (Hughes and Hall, 1988). Thus, the barrel chest and distended lower abdomen of *Monodelphis domestica* and *Macropus eugenii* result from the increase in the internal surface areas of the lung and gut. The alimentary tract of the newborn marsupial is complete but relatively undifferentiated. The stomach acts as a receptacle and a primary absorptive organ and exhibits a marked grade of differentiation G1 to G3 (Hughes and Hall, 1988). In newborn American opossums (Heuser, 1921) and native cats (Hill and Hill, 1955) the stomach consists of fundic and pyloric regions lined by a low columnar to cuboidal epithelium, with a wall of undifferentiated mesenchyme, without glands. In the neonatal *Macropus eugenii* the volume of the stomach is large and the epithelial lining consists of tall columnar vacuolated cells with evidence of functional diversity (Hughes and Hall, 1988). The duodenum is a relatively large thin-walled structure with its lining produced into prominent villous folds. The large intestine is of relatively small diameter compared to the small intestine (Sharman, 1973). The small intestine exhibits a parallel grade to the stomach G1 to G3. The absorptive areas in *M. eugenii* and *M. giganteus* are greater in length and diameter and possess a more pronounced brush order than that seen in *Sarcophilus harrisii*. The development of the other major abdominal viscera (liver, pancreas and mesonephros) is at a more advanced stage in *M. eugenii* than in *S. harrisii* and contributes to the elimination of the caudal taper seen in the G1 grade (Hughes and Hall, 1988). A functional mesonephros at birth is amongst the most unique features of marsupials. Morphological studies on the postnatal development of the kidney in *Isoodon macrourus* (Hall, 1987), *Didelphis virginiana* (Krause et al., 1979) and *Macropus eugenii* (Wilkes and Janssens, 1988) showed that at birth the mesonephros contains similar ultrastructural components (filtration slit pore, brush border, deep basal infoldings in cells lining the proximale tubule, mesangeal cells in the glomerulus) as the metanephric kidney. The metanephros in newborn marsupials is non-functional but clearly exhibits the series G1 to G3 (Hughes and Hall, 1988). At birth, the marsupial neonate has a well-developed digestive, respiratory and circulatory system, but retains its fetal excretory system with a fully functional mesonephric kidney and undifferentiated gonads and genitalia (Renfree et al., 2001).

The growth curves of the both marsupials examined indicate a slow growth rate during the early postnatal development and resemble each other. There are numerous studies on the growth of pouch young of various species of marsupials (Hill and Hill, 1955; Shield and Woolley, 1961; Sadleir, 1963; Kirkpatrick, 1965; Cothran et al., 1985; Poole et al., 1991; Trott et al, 2003). Although growth curves of marsupial young vary among species they are sigmoidal in shape and may be broadly described by the Gompertz equation (Cockburn and Johnson, 1988). Marsupials initially grow slowly, as seen in *Monodelphis domestica* and

Macropus eugenii, then increase in size rapidly, and then grow slowly again, until adult weight is reached. The rates of growth of 331 mammalian species were compared by Zullinger et al. (1984) by fitting the Gompertz growth model to published growth rates. In addition, Lee and Cockburn (1985) provided a more restricted analysis for a variety of marsupials. When compared to the curve for all mammals, it becomes obvious that marsupials grow generally slower than placentals.

It is difficult to find a good basis for comparison of rates of development. The age at which the eyes are fully open and the age that fur develops seem to be good measures, since they are frequently recorded with some degree of consistency (see appendix, table III). In comparison with the developmental rate in the four placental species, the morphological development of *Monodelphis domestica* and *Macropus eugenii* is rather slow. Janssens et al. (1997) recapitulate the postnatal development of *M.eugenii*. In the first 100 days of pouch life, the tammar young grows slowly, but growth hormone, cortisol and thyroid hormones (probably derived from the milk in the first weeks of pouch life) are present. The brain and kidney are growing more rapidly than the rest of the body and the brain reaches its maximum size relative to body weight at about 120 days. At about 140 days the eyes open and by 160 days the young can stand. In the second period of pouch life, from 120 days until the first pouch vacation at about 200 days, the kidney function matures and the young experiences a period of rapid growth that extends from about 150 to 220 days of age. The fur starts growing at 180 days of age. Also Russell (1982) describes a slow postnatal maturation in a variety of marsupials.

Green et al. (1988) have argued that the growth rate of marsupial young is limited by the supply of milk from the mother, an argument supported by the finding that if macropodid pouch young of different ages are exchanged, the growth of the smaller young is accelerated, while that of the larger young is reduced (Findlay, 1982; Findlay and Renfree 1984; Green and Merchant, 1988; Trott et al., 2003). Indeed, the composition of the milk of marsupials changes remarkably during the lactation period. The early milk of marsupials is dilute, containing about 10% solids. The content of solids in marsupial milk continually increases during lactation to average approximately 30 % towards the end of lactation (Green and Merchant, 1988). This change in milk solids is associated with a dramatic increase in total lipid content and a con-comitant large increase in the energy content of the milk, which is presumably associated with increased energy requirements of the marsupial young at this time (Janssens, 1997).

4.1.2 Placentalia

Placental mammals are characterised by birth occuring at different times during organogenetic profiles. In placentals a wide spectrum of altricial and precocial newborn, with many intermediate stages, are recognised. However, even the most altricial placental newborn is far more advanced than the most advanced marsupial newborn. Portmann (1951) distinguishes different types of ontogenesis. The primary precocial newborn of the Sauropsida (Crocodilia, Serpentes) stands opposite to the secondary precocial newborn of the Mammalia (Cetartiodactyla, Proboscidea) which is derived from the primary altricial newborn. As primary altricial newborns, neonates of altricial birds and primitive mammals (many Rodentia, Insectivora), which are born with closed sense organs, can be considered. The secondary altricial newborns of mammals (human) are characterised by open sense organs and locomotoric immaturity. Also Müller (1972) describes the precocial newborn as young born with open eyes and in contrast the altricial newborn as young born before eye opening. The phylogenetic way from the altricial to the precocial newborn consists in a prolonged gestation period and that the fetus is retained for long time in the uterus so that the eyes are already open when it is born.

In consideration of the previous definitions, the five placentals investigated can be assigned to primary altricial newborns (*Mesocricetus auratus, Suncus murinus*, and *Tupaia belangeri*) and secondary precocial newborns (*Cavia aperea, Macroscelides proboscideus*). The wide variety of ontogenetic birth stages in placental mammals is summarised in table III of the appendix. Within the Afrotheria, only the Afrosoricida have primary altricial newborns. The Xenarthra are heterogeneous, whereas the Bradypodidae have precocial newborns, the Dasypodidae seem to be altricial. Within the Euarchontoglires, the Scandentia and many Rodentia (Sciurognathi) have primary altricial neonates, whereas Primates, Lagomorpha and some Rodentia (Hystricognathi) are precocial born. Among the Laurasiatheria, the Eulipothyphla and most of the Carnivora (Fissipedia) are born in an altricial condition, whereas Chiroptera, Pholiodota, some Carnivora (Pinnipedia), Perissodactyla, and Cetartiodactyla have precocial offspring.

In placental mammals only two distinct phases during the development of young can be distinguished:

1. A period of intrauterine development varying, in different species, from 16 to 660 days.
2. Suckling period between birth and independency during which milk feeding proceeds and postnatal development will be finished.

Although many placentals produce altricial young, in all cases the young are more advanced in their development than marsupial young. Several authors describe a similar developmental stage in altricial newborns, mostly rodents and insectivores (Dryden, 1968; Müller, 1972; Hellwing, 1973; Grünwald and Möhres, 1974; Sterba, 1977 a, b; Jacobsen, 1982; Hertenstein et al., 1987; Burda, 1989; Bronner, 1992; Gusztak and Campbell, 2004). All altricial neonates are practicle naked, the skin nearly translucent and reddish-pink in colour, the vibrissae are well developed. The extremities are normally developed and provided with claws. The incisors, often present, are prominent but not in mutual contact. The head is comparatively big, the snout blunt and the nose convex. A bulged skin-fold represents the auricle; the external opening of the acoustical meatus seems to be closed. The eyes are closed and the newly born young are able to crawl unsteadily and to hold their head lifted for few seconds. The three newborns of *Mesocricetus auratus, Suncus murinus* and *Tupaia belangeri,* share these developmental characteristics and can be considered as typical altricial. Altricial placentals have no special adaptations or morphological features as described in the newborn marsupials. Generally the altricial young are placed in a nest, where they remain nursed by the female and undergo their postnatal development. Some species of desert rodents *(Otomys, Grammomys, Thallomys, Aethomys, Mystromys, Neotoma*, and *Baiomys*) use a method similar to marsupials, to carry their young around (Blair, 1941; Richardson, 1943; Mills and Hes, 1999). The young adhere to the teats, but not permanently as in marsupials. That strategy may be an adaptation to unfavourable habitats with few possibilities for nest sites, a mechanism to avoid predation of the young or as Müller (1972) assumes a perpetuation of original reproductive strategies.

Adult body size is a major factor in determining reproductive and life history strategies. Large size correlates with increased gestation length, reduced litter size and proportionally reduced maternal investment, slower growth rates, increased age at puberty, increased longevity and reproductive effort spread over several breeding seasons (Tyndale-Biscoe and Renfree, 1987). Large mammals must provide for their young during a prolonged growth period, and this extension of the period of parental care must be accomplished by extension of extrauterine development (marsupials) or intrauterine development (placentals). Large marsupials, as represented by the extant wombats and kangaroos have provided for this extrauterine growth phase by extended lactation and a large pouch (Russell, 1982). By contrast, large placentals prolong gestation and deliver precocious nidifugous young. These precocial neonates are typically furred at birth or soon after; their eyes typically are open at birth; and they mature comparatively rapidly in locomotor ability and other respects, achieving independence of their parents relatively early (Hill, 1992). The two placentals investigated *Cavia aperea* and *Macroscelides proboscideus* fit into this developmental pattern, and can be considered as typical precocial.

The internal organs of the placental newborns are much more advanced than that of marsupial neonates, but also show differences in the developmental degree ranging from altricial to precocial. Schultz et al. (2004) compare the organ systems of a marsupial, a altricial placental, and a precocial placental. In most newborn placentals a mesonephros as excretory organ does not occur (Ludwig, 1957; Witschi, 1962). In newborn *Rattus norvegicus, Mus musculus, Mesocricetus auratus and Macroscelides proboscideus* a well developed metanephros is used for excretion directly (Witschi, 1962; Schultz et al., 2004). The most advanced metanephros (convoluted nephrons, renal corpuscles) of the precocially born *Macroscelides proboscideus* is able to produce and excrete urine in a very effective way, which supports a high metabolic rate. Also the liver is well developed in placental newborns. A well developed sinusoid system runs through the liver parenchyma and leads blood from the larger blood vessels to the periportal fields (Schultz et al., 2004). Also the brain development proceeds fast in placental mammals, what may be a prerequisit for homeothermy. With the maturition of the central nervous system a coordinate movement is enabled and that is needed for shivering thermogenesis (Müller, 1972).

In comparison with the two marsupial species, the four placental mammals investigated have all high growth rates. The growth curves of *Mesocricetus auratus, Suncus murinus, Tupaia belangeri* and *Cavia aperea* are similar in that sense, that they undergo rapid postnatal development and reach adult weights much faster than marsupials. But the forms of the growth curves differ. Gaillard et al. (1997), using the flexible Chapman-Richards model for describing growth curves from birth to adulthood of 69 species of placental mammals, demonstrates that growth form among mammals is extremely variable. Some mammals have a decelerating growth rate from birth to maturity, whereas others have the more classical sigmoid curve, with peak growth rate occurring after birth. The s-shaped growth rate found in *Mesocricetus auratus* is confirmed by Lochbrunner (1956). Lochbrunner describes an initially relatively slow growth rate of 0.87 g/day until day 10 and a steep increase of the growth curve to 1.52 g/day until day 28, which can be explained by the beginning intake of solid food. The high growth rate of *Suncus murinus*, starting from birth, and the fast attaining of the adult weight is also reported by Dryden (1968). Two studies in Crocidurinae confirm a general high postnatal growth rate in the Soricidae (Hellwing, 1973; Grünwald and Möhres, 1974). The zig-zag-curve of the growth rate, found in *Tupaia belangeri*, was also reported by Hertenstein et al. (1987). The continuous rise of the growth rate, described from day 30, is explained by the leaving of the nest at this time and the start of feeding solid food. The variability in growth form is most likely linked to precocity at birth. Precocial mammals exhibit peak growth rate earlier than altricial mammals (Gaillard et al., 1997). The steady high growth rate in *Cavia aperea* confirms this statement. A similar growth pattern presents North (1999) for the domestic form of the guinea pig *Cavia porcellus*. As indicated by the growth

rates, also the morphological development proceeds rapid in placentals. The start of furring, within the first week of life is confirmed for all three altricial placentals investigated (Lochbrunner, 1956; Dryden, 1968; Hertenstein et al., 1987). The postnatal development, with regard to eye opening, age of furring and age of sexual maturity, represents table III in the appendix for a variety of mammals.

In general, small mammals have relatively higher postnatal growth rates than do large mammals. That means, small mammals attain adult weight at an earlier age than do large mammals. But, even among mammals of similar size, both metabolic rates and postnatal growth rates vary (Vaughan et al., 2000). Low growth rates in certain small mammals may be associated with unusually low metabolic rates, as is probably the case with the tenrec *Hemicentetes semispinosus* and possibly in *Mesocricetus auratus,* or with an adaptively long mother-young association. In general growth rates are determined by the energy content of the milk. Dryden and Anderson (1978) explain the rapid growth rate in *Suncus murinus* with the high energy content of the milk (17.5 % fat; energy 252 kcal /100g milk). Also the milk of *Tupaia belangeri* has a high content of fat (25.6 %) and protein (10.4 %) (Hertenstein, 1987). That can be explained with the unusual reproductive strategy ("absentee system", Martin, 1967) of *T. belangeri*, to leave the nest and to nurse the young only once every 48 hours. The advantage of this strategy could be that the female avoids drawing attention of predators to the nest. This "absentee system" is found also in elephant-shrews (Rathburn, 1979) and rabbits (Hertenstein, 1987), but is most extended in tupaias. Despite their immaturity, the young of *T. belangeri* have to be capable of own thermoregulation. The high fat content of the milk is essential for the heat production and the high protein content of the milk is used for a rapid growth. In contrast to the milks of *Suncus murinus* and *Tupaia belangeri*, the milks of the rodents investigated *Cavia aperea* and *Mesocricetus auratus* have less fat (5.7 % in *C. aperea*, 4.9 % in *M. auratus*) and a lower energy content (Robbins, 1983). That results in a slower growth rate in *M. auratus*, whereas *C. aperea* feeds in addition solid food from the first day of life. Postnatal growth rates reflect the life history of a mammal. High growth rates have evolved under such demanding conditions as stressful environments and short seasons for preparing for hibernation (Vaughan et al., 2000). Kleiman and Davis (1978) describe a need for high growth rates in young bats that must store fat for a long hibernation. Equally demanding conditions have selected for extremely rapid growth in most marine mammals (Cetacea, Pinnipedia) whose young must rapidly prepare for life at sea. The rapid growth of bats, pinnipeds and whales is again fascilated by the high-energy milk these animals produce (bats up to 25.8 % fat, pinnipeds up to 61.1 % fat; whales up to 40.9 % fat) (Robbins, 1983; Oftedal and Iverson, 1995; Vaughan et al., 2000).

4.1.3 Hypothesis on characteristics of a common ancestor

Research on amniotes has assumed that gradualistic change is the sole mechanism by which viviparity, placentation, and placentotrophy could have evolved (Blackburn, 1995). That gradualistic model incorporates three successive stages: a gradual increase in the duration of oviductal egg-retention, leading to viviparity; a gradual development in viviparous forms of a simple placenta that functions in gas exchange and water uptake; and a progressive reliance on the placenta as a means of supplying inorganic and organic nutrients for development, leading to placentotrophy (Blackburn, 1995). In this sense, maternal provision of post-ovulatory nutrients has preceded the evolution of viviparity in therian mammals (Blackburn, 1992). Lillegraven (1975) suggests that the common ancestor of marsupials and placentals was viviparous and bore virtually embryonic young; he hypothesises that viviparity did not evolve independently in marsupials and placentals. These groups of mammals seemingly diverged in the early Cretaceous, and compelling paleontological and anatomical evidence indicates an origin from a common ancestral stock. Several adaptations, such as pronounced muscularised forelimbs, claws and a developed locomotoric cortex, that ensure a rapid transition from the uro-genital opening to the teat have evolved in marsupials. Temporary closures of the eyes and ears in newborn marsupials guard against desiccation, and partial closure of the mouth ensures secure attachment to the teat and immovable jaws in which the dentary-squamosal jaw point (absent at birth) can develop. All these adaptations allow for a fast transition and a firm attachment to the maternal teat after birth, where the postnatal development proceeds slowly. Among placental mammals, the young are born partly in a highly altricial condition, but undergo a much faster postnatal development. Although not as poorly developed at birth as monotreme and marsupial young, they also share the closure of the eyes, ears, and mouth at similar stages as those of marsupials. In placentals that bear precocious young, these closures have no function, yet they still occur briefly during intrauterine development and perhaps represent a developmental stage inherited from a common ancestor with marsupials. This ancestor may have born rudimentary young after a brief gestation period, just as marsupials do today (Müller, 1972). From these similar beginnings, Tyndale-Biscoe and Renfree (1987) propose that placentals evolved reproductive characters associated with longer gestation, while marsupials evolved along a separate trajectory with an emphasis on lactation for early growth of the young.

4.2 Lung structure and lung development

At birth the organism has to shift gas exchange from the placenta to the lung. With the first breaths the airways are inflated and an air-tissue interface is created from which the blood can draw oxygen and from which carbon dioxide can leave the organism. From there on, the gas-exchange apparatus has to grow concurrently with body growth while constantly maintaining an adequate gas supply to the blood (Weibel, 1967).

The ontogenetic development of the lungs is best described for the human lung (Thurlbeck, 1975; Weibel, 1980; Burri, 1985), but seems to be similar within all mammals (Ten Have-Opbroek, 1981; Maloney, 1984). Pulmonary development proceeds through three distinct chronological periods: the embryonic, fetal and postnatal (Burri, 1985). The phases are related to the morphological stages the airway system undergoes during development. In the period of embryonic phase of development the trachea forms from a ventral groove in the foregut. At the caudal end of this groove, two epithelial buds grow laterally into the thickened mesenchymal mass derived from the splanchnopleura. From these embryonic structures, the right and left lung are formed: each epithelial bud is the anlage of a main bronchus, the splanchnopleural mesothelial leaflet gives rise to the visceral pleura, and the mesenchymal mass provides blood vessels and all connective tissue elements. Each bronchial tube grows in length and divides repeatedly by dichotomy; branching occurs at the terminal bud at the end of each branch and depends on an interaction between epithelium and mesenchyme. The result is a glandlike system of branched epithelial tubes. In agreement with the budding from the foregut, the epithelium of these tubes is derived from the endoderm; it is high columnar. The epithelial tubes grow and branch into the mesenchymal mass, always leaving a thin layer of mesenchyme between the tubes. At the same time that the bronchial tubes begin to form, blood vessels develop within the mesenchyme and form a network around them. In the human lung, about day 50 the embryonic period is considered to terminate and merge into the fetal one (Burri, 1985). During the fetal period, the lung undergoes three phases of development to reach a degree of functional maturity, which enables the organism to survive at birth: the pseudoglandular, canalicular, and terminal sac stage (Ten Have-Opbroek, 1981; Maloney, 1984; Burri, 1985). Through further growth and branching of the few bronchopulmonary segments, the future lung resembles more and more an acinar gland in the pseudoglandular phase. During this stage the airway tubes are lined proximally by high columnar cells, that distally decrease in height and are simple cuboidal at the periphery. The following canalicular stage is charcterised by three major events: the birth of the acinus at the beginning of this stage, the differentiation of the pulmonary epithelium in type I and type II pneumocytes and the development of the typical blood-air barrier, and the start of surfactant synthesis toward the end of this stage. At the end of the canalicular phase air spaces are

assumed to be formed down to the terminal sac region. The later air spaces are composed of the airways down to the last prospective respiratory bronchioles and of a cluster of wide, thin-walled, irregularly shaped saccules that have been called terminal air sacs. In the lung at birth, most of the airway generations are present; the peripheral ones, however, are short and greatly lengthen during postnatal development. The gas-exchange region is made of a few generations of wide and smooth-walled transitory ducts, of which the last ones open up into saccules. The pre- or postnatal development of alveoli is called the alveolar stage. Postnatal alveolarisation allows the lung to drastically increase its internal surface area and to adequately supply the rapidly growing organism with oxygen (Burri, 1985).

4.2.1 Marsupialia

In several species of marsupials at birth the lung parenchyma is at the early terminal air sac stage and the pulmonary vasculature is well developed with short air-gas diffusion distances (Baudinette et al., 1988b). The newborn marsupial lung is composed of a primitive system of branching airways which terminate in large blind air sacs (Fig. 106, Tab. 10). This pattern is typified by *Macropus eugenii* (Randall et al., 1984; Baudinette et al., 1988a; Ribbons et al., 1989; Runciman et al., 1996; Cehun, 1994), *Macropus rufogriseus (*Walker and Gemmell, 1983), *Setonix brachyurus* (Burri et al., 2003), *Isoodon macrourus* (Gemmell and Little, 1982; Gemmell, 1986), *Trichosurus vulpecula* (Gemmell and Nelson, 1988; Buaboocha and Gemmell, 1997), *Marmosa robinsoni* (Barnes, 1977), *Didelphis virginiana* (Bremer, 1904; Krause and Leeson, 1973, 1975), and *Monodelphis domestica* (Schmidt, 1996). According to the differences in the developmental degree of marsupial neonates, already mentioned in chapter 4.1, also the lungs of newborn marsupials show a graduation in the developmental degree from G1 to G3. In newborn dasyurids the lungs are much less developed (G1) than those of other marsupial species. The lungs of neonatal *Dasyurus hallucatus* (Gemmell and Nelson, 1988), *Dasyurus viverrinus* (Hill and Hill, 1955), *Sarcophilus harrisii* (Hughes and Hall, 1988), *Sminthopsis macroura* (Gemmell and Selwood, 1994), and *Sminthopsis douglasi* (Frappell and Mortola, 2000) consist of two sacs with no apparent divisions and only few septal crests protruding into the air space (see Fig. 106 a, b). However, the surface of the lungs is lined by capillaries. The lungs of the didelphides, including that of *Monodelphis domestica*, can be considered as developmental stage G2, with a poor developed bronchial system and large air sacs (see Fig. 106 c, d). The most advanced lungs of newborn marsupials (G3), with a more advanced bronchial system and partly smaller air sacs, are present in Perameloidea and Diprotodontia (includes *Macropus eugenii*) (see Fig. 106 e). The neonatal lungs of *Monodelphis domestica* and *Macropus eugenii* are relatively larger (a third and a fourth part of the whole body length) than that of the placentals examined (a sixth, a seventh, a fifth and a eighth part of the whole body length). Due to the highly immature

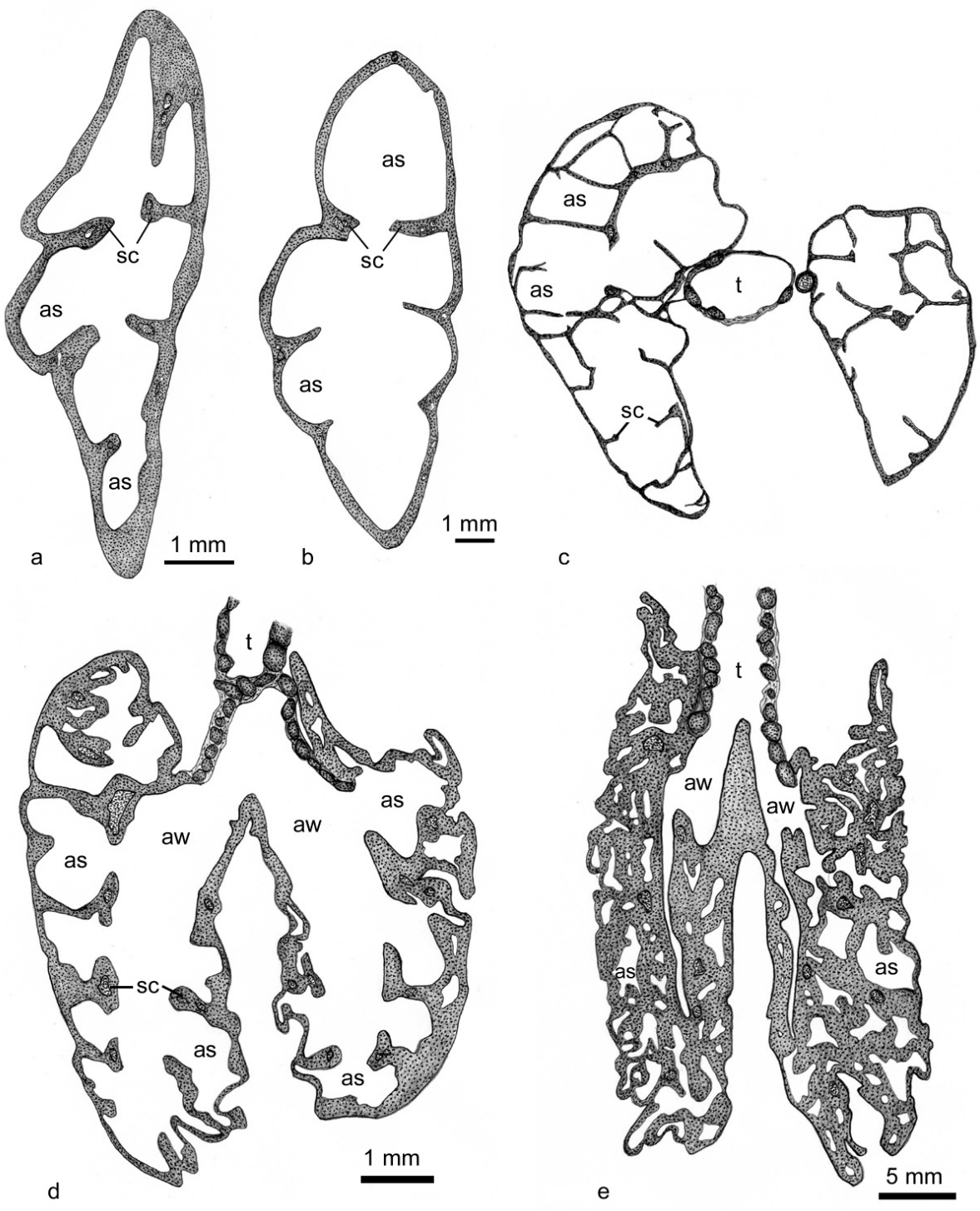

Fig. 106: Drawings of lungs from newborn *Sarcophilus harrisii* (a; redrawn from Hughes and Hall, 1988), *Dasyurus viverrinus* (b; redrawn from Hill and Hill, 1955), *Didelphis virginiana* (d; redrawn from Krause and Leeson, 1973), *Isoodon macrourus* (e; redrawn from Gemmell and Little, 1982) and a 12 days old *Marmosa robinsoni* (c; redrawn from Barnes, 1977).

lungs, a relatively large lung may be a strategy of the Marsupialia to provide the necessary surface area for the gas-exchange. The information concerning neonatal lung structure and postnatal lung maturation in marsupials are summarised in table 10. The lung development examined in *Monodelphis domestica* and *Macropus eugenii* and the data from the literature

Tab. 10: Survey of literature to the lung development of marsupial mammals.

Species	Developmental stage of lung at birth	Size of air spaces at birth	Septum at birth	Thickness of septa at birth	Thickness of blood-air barrier at birth	Formation of alveoli	Respiratory bronchioles and alveolar sacs	Reference
Marsupialia								
Marmosa robinsoni	air sac stage	large	double capillary	-	-	-	-	Barnes, 1977
Didelphis virginiana	air sac stage	-	double capillary	-	-	45; 45-85 days	60 days	Krause and Leeson, 1973, 1975
Monodelphis domestica	air sac stage	350-400 µm	double capillary	15-20 µm	500-1000 nm	28 days	28 days	Schmidt, 1996
Dasyurus viverrinus	air sac stage (two air sacs)	large	-	-	-	-	-	Hill and Hill, 1955
Dasyurus hallucatus	air sac stage (two air sacs)	large	-	-	-	-	-	Gemmell and Nelson, 1988
Sarcophilus harrisii	air sac stage (two air sacs)	large	-	-	-	-	-	Hughes and Hall, 1988
Sminthopsis douglasi	air sac stage (two air sacs)	395 µm (2 weeks)	-	-	-	-	-	Frappell and Mortola, 2000
Sminthopsis macroura	air sac stage (two air sacs)	large	-	-	-	-	-	Gemmell and Selwood, 1994
Isoodon macrourus	air sac stage	300-500 µm	double capillary	40-70 µm	-	40 days	-	Gemmell and Little 1982; Gemmell, 1986
Trichosurus vulpecula	air sac stage	330-600 µm	double capillary	-	-	39-113 dpn	-	Buaboocha and Gemmell 1997
Setonix brachyurus	air sac stage	-	double capillary	-	-	125 days	-	Burri et al., 2003
Macropus eugenii	air sac stage	150-350 µm	double capillary	35 µm	430-700 nm	70; 75 days	180 days	Runciman et al., 1996; Randall et al., 1984; Baudinette et al., 1988a; Ribbons et al., 1989
Macropus rufogriseus	air sac stage	175-195 µm	double capillary	27-33 µm	400 nm	-	-	Walker and Gemmell, 1983

assume that slow postnatal lung maturation is characteristic for marsupials. The beginning formation of alveoli at the age of 28 days in *Monodelphis domestica* is the earliest date reported for marsupials. *Macropus eugenii*, although further developed at birth, shows slower postnatal lung maturation (alveoli from day 65). That may be a typical pattern for macropodids, where postnatal development generally proceeds slower (Burri et al., 2003; Runciman et al., 1996). Several studies examined morphometric aspects in the developing marsupial lung (Runciman et al., 1998a, 1998b, 1999; Burri et al., 2003; Makanya et al., 2003). Runciman et al. (1998a) showed that the volume of capillaries and the surface area of the effective gas-exchange tissue is greater in the newborn rat than in the newborn Tammar wallaby. During marsupial postnatal development, capillary and endothelial volumes as well as the capillary and airspace surface areas show highest rates of increase during the alveolar stage. The pulmonary diffusion capacity increased gradually to adult values, comparable to those of placentals (Burri et al., 2003). One characteristic of the newborn *Monodelphis domestica* and *Macropus eugenii* (Runciman, 1994; Cehun, 1994) are the large, nucleated erythrocytes in the blood. The respiratory properties of neonatal blood of *Trichosurus vulpecula* and *Macropus eugenii* were investigated before by Tibben et al. (1991) and Calvert et al. (1994). They report a low-oxygen-affinity blood, a low Bohr Effect, and multiple haemoglobin types. The multiple haemoglobin types and the large nucleated erythrocytes establish this blood as being embryonic type and it is likely that the properties of the blood in the immediate postnatal period reflect the function of the blood before birth (Calvert et al., 1994). Baudinette et al. (1988) showed that the oxygen affinity of the blood was much lower in the early pouch young than in the adult. During the postnatal development the erythrocytes lose their nuclei and the respiratory properties of the blood change and resemble that reported for placentals (Bland and Holland, 1977). The changes in lung structure throughout pouch life probably reflect the increased respiratory requirements of the developing young. Although the time sequence of lung development of marsupials differs from that in placentals, the adult form of the lung in both animal groups is similar in structure (Gemmell, 1986). In all studies on marsupials so far, it is clear that lung development in marsupials and placentals follows the same general pattern of mammalian lung development, but differs in the developmental rate.

4.2.2 Placentalia

The lung of most altricial placental mammals at birth is structurally immature yet functional as a gas exchanger, and the septation process which leads to the formation of alveoli occurs primarily during early postnatal life (Baudinette et al., 1988). In altricial species, the lungs are characterised by a well developed bronchial system, which terminates in small air sacs. Typical altricial placentals, in terms of lung development, are *Mesocricetus auratus* and

Suncus murinus, but also other Soricidae (Foresman, 1994), *Mus musculus* (Ten Have-Opbroek, 1981), and *Rattus rattus* (Weibel, 1970; Burri, 1974; Brody and Vaccaro, 1979) (see Tab. 11). In these species the postnatal lung development is rapid and the formation of alveoli starts in *Mesocricetus auratus* at the age of 2 days, in *Suncus murinus* and *Rattus rattus* at the age of 4 days, and in *Mus musculus* at the age of 5 days. Although *Tupaia belangeri* is obviously altricial in its external appearance (closed eyes, hairless) it is advanced in its neonatal lung structure and represents an immediate stage. Beside small air sacs, the lung has alveoli and associated structures (respiratory bronchioles, alveolar sacs) already at birth. In respect of lung development, *Tupaia belangeri* appears to be precocial. That may be a prerequisit for the previously described “absentee system” in tupaias, which demands a high metabolic rate of the young to cope with the thermoregulatory needs while the mother is absent.

Numerous placental mammals reach the alveolar stage in utero and already have alveoli at birth. The lung of these precocial placentals is composed of a well developed bronchial system, which terminates in respiratory bronchioles, alveolar ducts and alveolar sacs. The entirety of the numerous small alveoli provides a large gas exchange surface area. The newborn *Cavia aperea* and *Macroscelides proboscideus* are two typical representatives of this precocial lung type. Also the newborns of *Cavia porcellus* (Lechner and Banchero, 1982), *Oryctolagus cunniculus* (Snyder et al., 1992), *Felis cattus* (Kikkawa et al., 1971; Mercurio and Rhodin, 1984), *Canis familiaris* (Boyden and Tompsett, 1961), *Sus domestica* (Winkler and Cheville, 1984, 1985), *Bos taurus* (Castleman and Lay, 1990), *Ovis aries* (Maloney, 1984; Davies et al., 1988; Docimo et al., 1991), *Capra hircus* (Kahwa et al., 2000); *Macaca fascicularis* (Hislop et al., 1984), and *Homo s. sapiens* (Engel, 1962; Weibel, 1970; Brody and Vaccaro, 1979; Langston et al., 1984; Zeltner and Burri, 1987; Zeltner et al., 1987) are born at the alveolar stage of lung development (see Tab. 11). The growth of alveoli, accompanied by additional formation of new units, proceeds in the postnatal period and especially in man (Moore, 1977), and presumably other larger species, this process occurs over a much longer period than in small placentals (Baudinette et al., 1988).

Morphometric studies on rat lung growth found that the formation of alveoli, occuring between day 4 and 10 results in a five-fold increase in gas exchange surface area (Weibel, 1967; Burri, 1974; Burri et al., 1974). It was found that the alveolar surface area increased by a factor of 2.6 whereas the lung volume increased by only 60 % during this period. The volume of capillaries per unit alveolar surface dropped by more than 50 %, although the total capillary volume of the lungs increased slightly. These findings suggested that profound changes in pulmonary architecture must occur in the rat lung between the 4th and the 10th day of postnatal growth. A detailed analysis revealed that this period was characterised by the formation of alveoli and of single capillary networks in the interalveolar septa (Weibel,

Tab. 11: Survey of literature to the lung development of placental mammals.

Species	Developmental stage of lung at birth	Size of air spaces at birth	Septum at birth	Thickness of septa at birth	Thickness of blood-air barrier at birth	Formation of alveoli	Respiratory bronchioles and alveolar sacs	References
Placentalia								
Sorex vagrans	air sac stage	small	double capillary	-	-	-	-	Foresman, 1994
Sorex cinereus	air sac stage	small	double capillary	-	-	-	-	Foresman, 1994
Mus musculus	air sac stage	small	double capillary	-	-	5 dpn	-	Ten Have-Opbroek, 1981
Mesocricetus auratus	air sac stage	small	double capillary	-	-	2 dpn	-	Ito and Kanisawa, 1990
Rattus rattus	air sac stage	80-120 µm	double capillary	12 µm	thin	4; 4-13; 5-10 days	10-13 days	Weibel,1970; Burri, 1974; Brody and Vaccaro, 1979
Cavia porcellus	alveolar stage	73 µm	single capillary	-	-	before birth	before birth	Lechner and Banchero, 1982
Oryctolagus cunniculus	alveolar stage	-	single capillary	-	-	before birth	before birth	Snyder et al., 1992
Felis cattus	alveolar stage	-	single capillary	-	560 nm	before birth	before birth	Mercurio and Rhodin, 1984
Canis familiaris	alveolar stage	-	single capillary	-	-	before birth	before birth	Boyden and Tompsett, 1961
Sus domestica	alveolar stage	30-40 µm	single capillary	8-12 µm	250 nm	before birth	before birth	Winkler and Cheville, 1984, 1985
Bos taurus	alveolar stage	-	single capillary	-	-	before birth	before birth	Castleman and Lay, 1990
Capra hircus	alveolar stage	-	single capillary	-	-	before birth	7 days	Kahwa et al., 2000
Ovis aries	alveolar stage	-	single capillary	2.55 µm	-	before birth	before birth	Maloney, 1984; Davies et al., 1988; Docimo et al., 1991
Macaca fascicularis	alveolar stage	54 µm	single capillary	-	-	before birth	before birth	Hislop et al., 1984
Homo s. sapiens	alveolar stage	50 µm	single capillary	15-25 µm	200-500 nm	before birth	before birth	Weibel, 1970; Brody and Vaccaro, 1979; Zeltner and Burri, 1987

1970). Several morphometric studies on precocial born placentals reveal that they undergo a large increase in pulmonary gas exchange area, associated with alveolar development, in utero (Alcorn et al., 1981; Hislop et al., 1984; Winkler and Cheville, 1985; Collins et al., 1986; Zeltner et al., 1987; Davies et al., 1988; Castleman and Lay, 1990; Docimo et al., 1991). Thus, just prior birth, the precocial lung has a well-developed airway system and alveolar network, in preparation for postnatal gas exchange. The results suggest that the active precocial newborns require an alveolar lung, but in the postnatal period functional needs increase only moderately.

4.2.3 Comparative aspects of the mammalian lung structure

The mechanisms of lung development seem to be the same in all mammals, only the developmental rates differ. The developmental degree in terms of lung development of all seven mammalian species investigated are summarised in figure 107. The earliest stage of

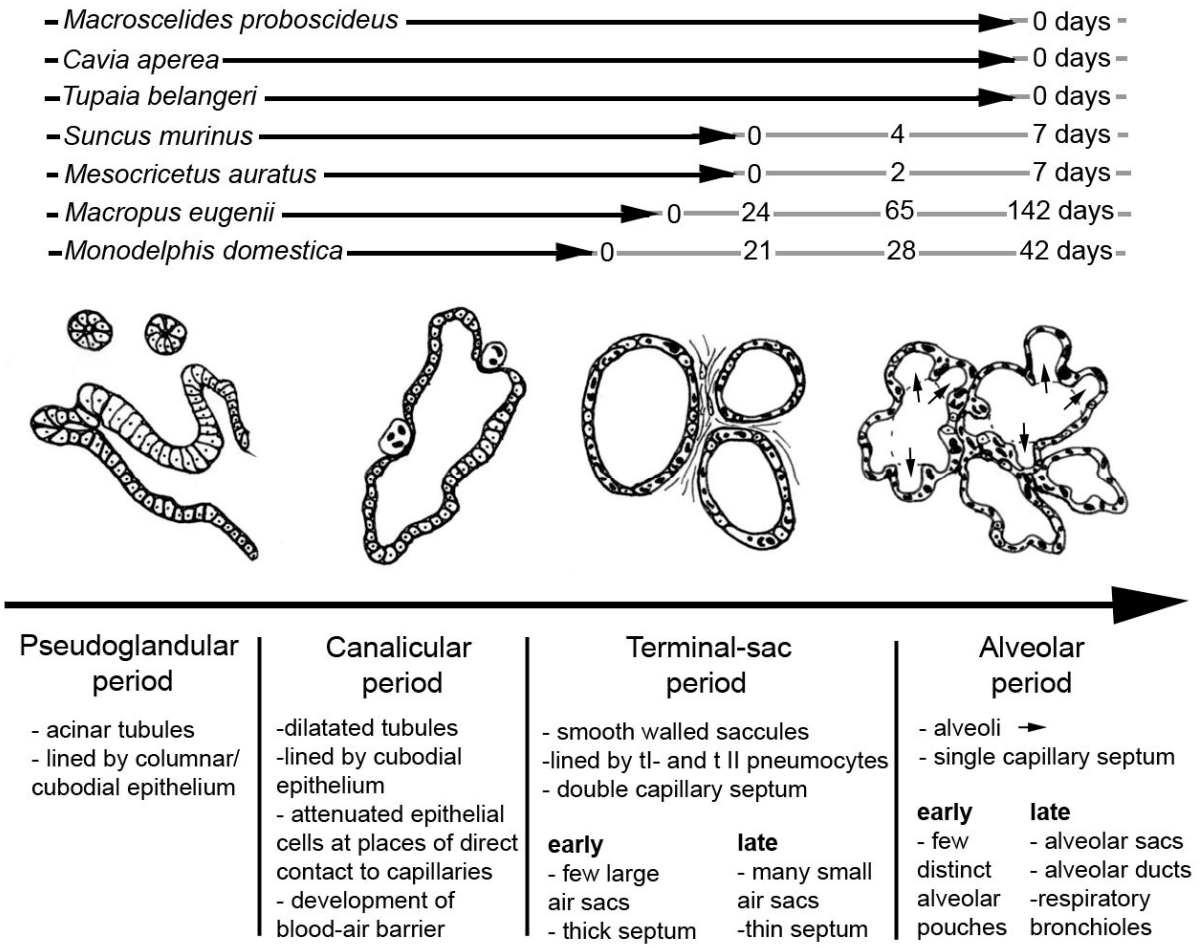

Fig. 107: Summary diagram of lung development in the seven mammalian species examined. For each species a timeline shows the developmental degree at birth (arrows) and the main postnatal stages of lung development (grey line with age).

development of the lung at birth is the late canalicular or more probably the early terminal sac stage of lung development, because a gas exchange area consisting of a capillary septum with a blood-air barrier must be present. This developmental stage is realised in newborn Marsupialia and Monotremata (see 3.2.4). During postnatal development, the large air sacs become subdivided and separated by septal crests and smaller air sacs can be found. For example, the marsupials *Monodelphis domestica* and *Macropus eugenii* reach the small air sac stage, as found in the newborn *Mesocricetus auratus* and *Suncus murinus*, with 21 and 24 days respectively. The advanced developmental birth stage of *Tupaia belangeri*, *Cavia aperea* and *Macroscelides proboscideus,* with respiratory bronchioles and alveolar sacs, can be found in *Monodelphis domestica* at 42 days, in *Macropus eugenii* at 142 days, in *Mesocricetus auratus* and *Suncus murinus* at 7 days. Finally, the adult lungs of all seven mammals examined are similar in structure.

Runciman (1994), Schmidt (1996), and Krause and Leeson (1973) described the same structural changes (thinning of septa, subseptation of air sacs) as found in the both marsupials examined. The general principles of the formation of alveoli, by a rapid outgrowth of single capillary septa from the double capillary septa, and the structural transformation from double capillary in single capillary septa explains Burri (1974) in detail.

Ultrastructurally, the lungs of all the species examined resemble each other. All have ciliated and non-ciliated (Clara) cells in the terminal bronchioles. The important role that cilia play in surface defence mechanisms of the respiratory tract is well recognised (Wright et al., 1983; Iovannitti et al., 1985; Ito and Kanisawa, 1990; Gulisano et al., 1995). They move secretions and trapped airborne particles toward the pharynx (Cross and Mercer, 1993). The nonciliated epithelial cells of the distal pulmonary airways (bronchioles) were described by Clara (1937). Three roles in normal function of adult lung have been proposed for this cell type: (1) secretion of airway-lining material, (2) metabolism of xenobiotic compounds, and (3) progenitor cell for bronchiolar epithelial regeneration (Plopper et al., 1983). The squamous type I pneumocytes and the cuboidal type II pneumocytes are characteristic for the respiratory epithelium in all mammals (Thurlbeck, 1975). Both cell types were already present at birth in all species examined. The type I pneumocytes make up only 7 % of the lining cells of the alveoli, yet they cover 95 % of the surface area. The thinness of this layer (0.15 µm) is an important factor in efficient gas exchange (Cross and Mercer, 1993). This epithelial layer is one component of the blood-air barrier. All mammals examined in this study have a trilaminar blood-air barrier, composed of epithelial type I pneumocytes, capillary endothelial cells and a fused basal lamina of both cell types. This structure is constant in all mammals and the extreme attenuation of the endothelial and epithelial surfaces fascilitates efficient exchange of oxygen and carbon dioxide via short diffusion distances (Burri, 1974; Winkler and Cheville, 1985; Gemmell and Nelson, 1988; Cross and Mercer, 1993; Runciman,

1994). The attenuated type I pneumocytes are particularly susceptible to damage and yet are not capable of dividing and replacing themselves following injury. Repair and routine replacement is carried out by type II pneumocytes, which switch from their secretory activity to divide and differentiate into type I pneumocytes (Adamson and Bowden, 1975). The type II pneumocytes are cuboidal cells that occupy crevices in the alveolar surface, interspersed among the type I cells and attached to them by junctional complexes (Bucher and Wartenberg, 1989; Cross and Mercer, 1993). It has been established that the type II pneumocytes are secretory and are the source of pulmonary surfactant (Buckingham and Avery, 1962; Bensch et al., 1964; Hitchcock, 1980; Snyder and Magliato, 1991). In all stages of all species, ultrastructurally examined in this study, the type II pneumocytes are characterised by cytoplasmic inclusions referred to as lamellar bodies. These structures have been implicated in surfactant metabolism, as places of synthesis and secretion of surfactant (Wang et al., 1971; Adamson and Bowden, 1973; Hitchcock, 1980). Some studies showed the process of secreting such lamellar bodies and surfactant into the alveolar space, as seen several times in my investigations (Bensch et al., 1964; O'Hare et al., 1971; O'Hare and Sheridan, 1970). Surfactant is a surface-active material which coats the alveolar surface, decreases the surface tension, and thus stabilises the alveoli or air sacs against collapse during exhalation and reduces the amount of energy required to inflate the lungs during inhalation (Hitchcock, 1980; Cross and Mercer, 1993). The presence of this pulmonary surfactant is essential for normal pulmonary function and a mature surfactant system at birth is a necessary prerequisit for air breathing. Its absence is an important etiologic factor in the respiratory distress syndrome in humans (Hitchcock, 1980). For that reason type II pneumocytes produce surfactant towards the end of gestation (Kikkawa et al., 1971; Burri, 1985) and, despite the short gestational period, marsupials are born with a mature surfactant system (Baudinette et al., 1988b, Ribbons et al., 1989; Runciman, 1994; Cehun, 1994). Besides, Condon et al. (2004) states that the surfactant protein A (SP-A) acts as a hormone that signals the initiation of parturition. Biochemical analyses indicate that surfactant is a complex mixture of phospholipids, neutral lipids, proteins, and possibly carbohydrates; the major phospholipid present is dipalmitoylphosphatidylcholine (DPPC) (Orgeig and Daniels, 2001). Surfactant seems to be phylogenetic very old, despite temporal differences and vastly different lung morphologies, birth strategies and phylogenies, the overall development and maturation of the pulmonary surfactant system is highly conserved amongst the amniotes (Johnston and Daniels, 2001; Orgeig et al., 2003; Sullivan et al., 2003).

The comparison of the monotreme, marsupial and placental lung structure shows that the mammalian lung is highly conserved and that the differences in the neonatal lungs can be explained simply by different developmental rates. All mammals have four pulmonary lobes in the right lung, whereas the number of pulmonary lobes in the left lung can vary (Nakakuki,

1980). The pulmonary interior (bronchial / bronchiolar epithelium, respiratory epithelium) and the principles of septal transformation are the same in all mammals. In general, the pulmonary structure at birth depends on the extent of intra-uterine development (Engel, 1962). Thus, the high degree of immaturity in the lung of newborn marsupials reflects the general immaturity at birth and results from a short intra-uterine development, nourished via a less efficient chorio-vitelline placenta. In contrast, placentals have a more advanced lung at birth, reflecting the longer intra-uterine development, nourished via a more efficient chorio-allantoic placenta. In comparsion with the monotreme lung, it becomes obvious, that marsupials and monotremes represent the original developmental degree of the mammalian lung at birth, whereas the placental newborns are derived to a lesser (altricial) of higher (precocial) degree.

4.3 Metabolism during the early postnatal period

When comparing the metabolic rate of adult vertebrates the convention has been to use the concept of a standard metabolic rate (SMR). This is normally measured as the oxygen consumption of a resting, post-absorptive adult in a thermoneutral environment with a normal body temperature for the particular species (Hulbert, 1988). The SMR of adult animals is related to body weight (W) in an allometric manner, that is, $SMR = a*W^b$ (Kleiber, 1961). The exponent (b) was for long time assumed to be 0.75 in all mammals (Kleiber, 1961; Dawson and Hulbert, 1970; Poczopko, 1971; Martin, 1980; Koteja, 1987), but is more recently subject of discussion. Hayssen and Lacy (1985) and Lee and Cockburn (1985) took into consideration, that adult marsupials have a level of standard metabolism that is approximately 70 % of the rate for adult placental mammals and presented two different equations for marsupial and placental mammals (see chapter 3.3). Furthermore, White and Seymour (2003) stated a scaling exponent of 0.667 and several studies discussed the suitability of the 3/4 or 2/3 power scaling (Bokma, 2004; Kozlowski and Konarzewski, 2004; Savage et al., 2004; White and Seymour, 2005). Under consideration of body size, lifespan, and energy metabolism, Speakman (2005) describes, that the scaling exponent for the relationship of SMR to body mass lies somewhere between 0.66 and 0.8. Nevertheless the “metabolic size allometry” is described approximately by the “mouse-elephant-curve” (Fig. 108 a). It explains the fact, that smaller mammals, due to their greater surface-to-volume ratio, loose more heat to the surrounding than larger species. Therefore, they need a higher endogenous heat production to maintain a constant body temperature (“surface law”). Given their high specific metabolic rate, it is not suprising that small mammals have evolved special adaptation strategies to avoid a discrepancy between energy demand and energy supply.

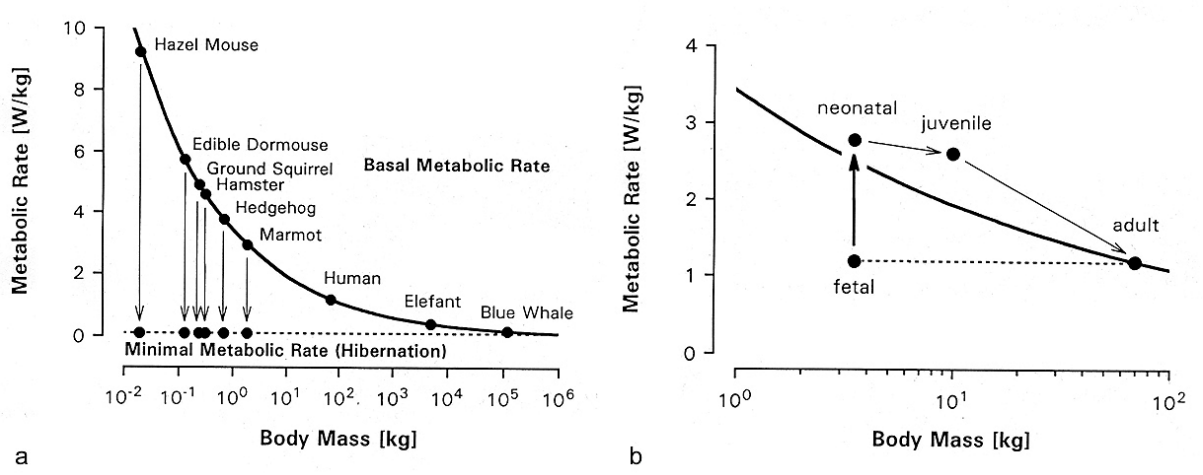

Fig. 108: The “switching-off of Kleiber’s rule” in hibernating and fetal mammals: (a) In mammalian hibernators, the turnover rate is lowered to a minimal level which equals the specific standard metabolic rate of the very largest mammals and, thus, suggests a common limit to metabolic reduction in mammals. (b) In the mammalian fetus, the specific metabolic rate equals the adult level (the fetus “is like an organ of its mother”), and the value to be expected from body mass is reached only after birth (data from humans). From Singer (2004).

Examples are the hibernators which escape the seasonal scarcity of food by cooling down to body temperatures near the freezing point (Singer, 1989). As described by Geiser (1988) and Heldmair and Ruf (1992), they maintain during the hibernating state a relatively uniform low specific metabolic rate of around 0.1 W/kg, which equals the specific standard metabolic rate of the largest mammals, such as blue whale and elephant, attained by body mass alone (Fig. 108 a). Referring to the temporary “switching off” of metabolic size allometry in hibernation, there can be found a parallel to this phenomenon in the peripartum (Singer, 2001, 2004). The mammalian fetus, as an exception to the overall “surface law”, exhibits the same specific metabolic rate as the mother, and reaches the metabolic level (or even somewhat higher) to be expected from its own body size only after birth (Fig. 108 b; Rahn, 1982; Singer, 2001). The results of the indirect calorimetric measurements in this study reveal that an increase of the metabolic rate after birth is valid only for placentals. The two marsupial species show a low (fetal) metabolic rate also postnatal. This leads to the assumption that the pouch young marsupial, just like the fetus, is also an exception to the “surface law”.

Singer (2001, 2004), Frappell et al. (1991, 1992), Mortola and Lanthier (1996), and Mortola (2001) explained that the inactivation of metabolic size allometry is not only an adaptation to the limited energy supply during the gestational period, but may also act as an additional protective factor against the risk of hypoxia involved in mammalian birth.

4.3.1 Placentalia

For the newborn, the birth is connected with a drastic change in the environment, from the constant warm uterus to the usually colder outside world. One would predict that newborns, because of their smaller size and their greater propensity for heat loss, should have higher weight-specific standard metabolic rates than adults (Mortola, 2001). In the early postnatal phases, the surge of catecholamines associated with the stress of parturition, and the sudden exposure to hyperoxia, are additional factors increasing neonatal metabolism (Clements et al., 1998; Mortola, 2001; Singer, 2001). And indeed, the placental neonates of *Mesocricetus auratus*, *Suncus murinus*, *Tupaia belangeri* and *Cavia aperea* have higher mass specific oxygen consumptions (VO_2) than the adults. This pattern is essentially confirmed for numerous mammalian species (for review, see table 12 and Mortola (2001). As reasons for the rise in standard metabolic rate, an increase in energy requirements of various tissues, such as brain, skeletal musculature and gastro-intestinal tract are discussed (Adamson et al., 1969). In fact, in many newborns VO_2 is also larger than in the same-size adults of other species. That is the case in *Suncus murinus*, *Tupaia belangeri* and *Cavia aperea*, where the VO_2 tops the mass specific expected values for adults. The postnatal metabolic curves of *Suncus murinus*, *Mesocricetus auratus*, and *Cavia aperea* are confirmed by other studies (Dryden et al., 1974; Mortola, 1991; Künkele, pers. comm.).

Metabolic rates are also dependent of the energy source, the animals use (Wilmer et al., 2000). In that context, the high metabolic rate of the neonatal *Suncus murinus* can be explained by the high energy content (17.5 % fat) of the milk. In contrast the neonatal *Mesocricetus auratus*, with the same body size, has a comparatively low metabolic rate, due to the lower energy content (4.9 % fat) of the consumed milk.

The data points of several neonatal species are plotted in figure 109. As is the case for the adults, among newborns mass-specific VO_2 is not a constant; rather, it decreases progressively in the larger species. But the interspecies differences are less pronounced than those among adults and the allometric exponent of the metabolic curve in newborns (0.92) is much higher than that of adults (Mortola, 2001). Although at birth metabolic rate is likely to increase in all placental species, the changes during the following hours and days can be quite varied. Many small altricial species, like the mouse and many other rodents, have a low, mass-specific metabolic rate in the first postnatal days, but substantially increase VO_2 during the first 1-2 weeks (Waldschmidt and Müller, 1988). Other species, like *Mesocricetus auratus, Suncus murinus, Tupaia belangeri*, and *Cavia aperea* but also *Dasypus novemcinctus* (Bagatto et al., 2000) drop VO_2 during the first week of life. In many precocial species, like *Equus caballus* (Stewart et al., 1984; Ousey et al. 1991) and *Sus domestica* (Noblet and Dividich, 1981), the drop of VO_2 to adult levels occurs within the first 24 hours of life. Also the precocial born *Cavia aperea* dropped its VO_2 during the first four days, after that

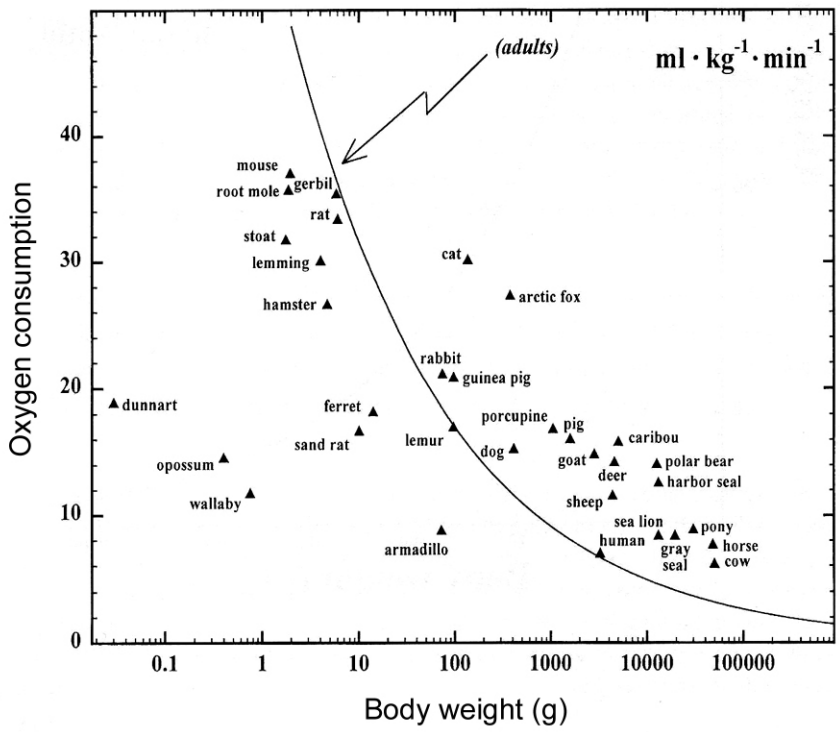

Fig. 109: Oxygen consumption in newborn mammals, plotted as a function of the newborn body mass. Average values of VO_2/kg of newborns of several species. Continuous line is the adult line. The three species at lower left are all marsupials. From Mortola (2001).

time the changes in metabolic rate are dependent only on the weight increase of the young.

4.3.2 Marsupialia

In marsupials the rule of the rise in metabolic rate at birth and the described postnatal metabolic changes, do not seem to be valid. The two neonatal marsupials *Monodelphis domestica* and *Macropus eugenii* have low mass specific standard metabolic rates similar to adults. They reach values, which amount only 22.4 % of the value to be expected from body weight. From figure 108 b, it can be assumed, that marsupials maintain the fetal metabolism also extra-uterine after birth. That would confirm the point of view of Zeller et al. (2004), who describes the marsupial neonate as “body appendage” of the mother. Hulbert (1988) concludes from metabolic measurements of pouch young *Macropus eugenii*, that they are born with a low level of energy metabolism (similar to that of reptiles) and that they attain at some time during their development a “mammalian” (specifically “marsupial”) level of energy metabolism. All that allows to suppose that Marsupialia have developed an alternate strategy to save energy in respect to their highly immature offspring. The marsupial young is adapted to save energy by getting all its energy needs from the mother, just as a placental fetus.

The postnatal metabolic curves of *Monodelphis domestica* and *Macropus eugenii* are

confirmed by other studies (Setchell, 1970, 1974; Baudinette et al., 1988a; Singer, 1998, 2001). During the postnatal development of *Monodelphis domestica* and *Macropus eugenii*, the SMR of the young marsupials increases slowly, with a significant rise in metabolic rate between six and eight weeks in *Monodelphis domestica* and starting from day 65 in *Macropus eugenii*. This increase in metabolic rate may be correlated with the more mature lung structure and with dietary changes of the young marsupials at this time. In *Monodelphis domestica* the young are weaned at the age of 50 days. With the weaning a dietary change from milk to solid food takes place and a higher metabolic rate may result. In contrast to this, the second marsupial examined *Macropus eugenii* has a longer lactation period (270 days). However, significant changes in the milk composition occur through lactation in the tammar wallaby. The concentration of total lipids in the milk of marsupials is low in the early stages of lactation and shows a dramatic incease in the total lipid of milk as lactation proceeds (Green and Merchant, 1988). The relative composition of milk lipids also changes extensively throughout lactation. In *M. eugenii* over 90 % of milk fats are in the form of triglycerids at day 70, whereas in earlier milk this proportion is only about 75 % or less (Green et al., 1983). In the early milk of *M. eugenii* palmitic acid (C16:0) is the predominant fatty acid representing about 50 % of the total triglyceride fraction (Green and Merchant, 1988). That could be associated with the high requirement of surfactants in the developing lung, because palmitic acid is known to be important in the synthesis of surfactant (Baudinette et al., 1988b). Although surfactant is present already at birth, it is removed and synthesised through the whole life time. Especially in the early postnatal periode, when the subseptation of the lung parenchyma leads to a surface enlargement of the gas exchanging structures, a lot of additional surfactant is needed to cover and stabilise the air spaces. However, about day 50 to 70, right at the time of the metabolic increase in *M. eugenii*, the levels of palmitic acid decline and are coincidentally replaced by oleic acid (C18:1), so that in the later stages of lactation oleic acid represents over 50 % of the milk triglycerids and palmitic only 20 % (Green and Merchant, 1988). The rise in the metabolic rate at this time argues for an increase in the nutritive value of the tammar wallaby milk.

The pattern of low metabolic rate at birth, slow increase of metabolic rate during postnatal development, and late attaining of adult metabolic rate was also observed in other marsupial species and seems to be typical for marsupials. For example, the adult metabolic rate is attained in *Setonix brachyurus* between day 100 and 119 (Shield, 1966), in *Macropus rufogriseus* at day 100 (Loudon et al., 1985), in *Didelphis virginia* after day 95 (Reynolds, 1952; Farber et al., 1972), in *Bettongia gaimardi* at day 84 (Rose and Kuswanti, 2004), and in *Dasyuroides byrnei* already present at day 58 (Geiser et al., 1986). Singer (2004) states, that the low specific metabolic rate of marsupials is a precondition of efficient growth and tissue aerobiosis in spite of extreme immaturity. Obviously, adaptive suppression of elevated

metabolism in organisms of small size results in a dramatic improvement of oxygen supply (Singer, 2004). That can be understood as energy saving mechanism.

4.3.3 Development of thermoregulation

In order to maintain a constant body temperature in cold environments, mammals minimise heat loss (i.e. behavioural thermoregulation) and, if this is not enough, increase heat production to balance heat loss (physiological autonomous thermoregulation) (Hulbert, 1988). However, that depends on the metabolic capacity of the animal. A review of the literature to the metabolic and thermoregulatory development in marsupial and placental mammals is presented in table 12. In marsupials, initially the thermogenic response is small, but gradually the maximal thermogenesis increases until it is relatively similar to that of the adult (Setchell, 1974; Geiser et al., 1986; Gemmell and Cepon, 1993). During this period of gradual attainment of thermogenic capacity, hair growth begins and by the time full thermogenic capacity is reached a complete pelage is present. Full thermogenic capacity is reached at the same time, when the adult metabolic level is attained. The nature and the site of the endothermic thermogenesis are not clear, but there may be both shivering and nonshivering components (Hulbert, 1988). In contrast, in placental mammals the thermogenic capacity is attained much earlier during postnatal development. A review of the large literature on metabolic responses of isolated young leaves no doubt that the majority of altricial placental small mammals that have been studied show signs of activating thermogenesis in response to declining ambient temperature at an early age (Hill, 1992). Isolated *Neotoma floridana* (Webb and McClure, 1989), *Sylvilagus floridanus* (Gates, 1974), *Gerbillus perpallidus* (Waldschmidt und Müller, 1988), *Microtus arvalis* (Gelineo, 1962), *Rattus rattus* (Taylor, 1960), and *Oryctolagus cuniculus* (Edson and Hull, 1977) show increases in metabolism in response to lowered ambient temperature within a day of birth. *Mus musculus* show increase within one to two days (Hill, 1970), and *P. maniculatus* (Chew and Spencer, 1967) and *Lemmus lemmus* (Hissa, 1968) exhibit such responses within two days. However, there are exceptions. *Mesocricetus auratus*, for example, seem to show no evidence of endogenous thermoregulatory responses to cold until 7-10 days old (Hissa, 1968; Rink, 1969). This trait is possibly related to their exceptionally short gestation time compared to other placentals (Hill, 1992) or with their special life strategy as burrowing mammals with food storage, which enables a permanent warming of the offspring in the nest. The full thermogenic capacity is reached in altricial species later and is generally correlated with the development of an insulating fur. It needs to be mentioned, that the thermoregulatory response of altricial offspring was generally measured at artificial conditions, such as isolated young and without or with artificial nests. In nature, the nestlings huddle together in a nest and thus can maintain a higher body temperature with lower metabolic rates than isolates

Tab. 12: Survey of literature to the metabolic and thermoregulatory development in marsupial and placental mammals.

Species	Neonatal mass specific standard metabolic rate (ml O_2*g^{-1}*hr^{-1})	Adult mass specific standard metabolic rate (ml O_2*g^{-1}*hr^{-1})	Attaining of adult metabolic rate (day)	Covering with pelage (day)	Onset of thermoregulation (day)	Full thermogenic capacity (day)	Reference
Marsupialia							
Monodelphis domestica	1.03	0.98	56	42	-	-	Singer, 1998; this study
Didelphis virginiana	0.85-0.90	0.5-0.6	after 95	60	-	70	McCrady, 1938; Farber et al., 1972
Dasyuroides byrnei	-	0.76	before 58	76	85-90	105-106	Geiser et al., 1986
Sminthopsis douglasi	0.9; 1.08	1.63	-	-	-	-	Mortola et al., 1999; Frappell and Mortola, 2000
Trichosurus vulpecula	-	0.32	-	117	130-133	140-143	Gemmell and Cepon, 1993
Potorous tridactylus	-	0.46	-	90	-	103	Gemmel et al., 1987
Bettongia gaimardi	0.46 (day 42)	0.65	84	84	70	84	Rose and Kuswanti, 2004
Setonix brachyurus	0.72	0.3	100-119	130-145	100	120	Sharman, 1959; Shield,1966
Macropus rufogriseus	-	-	100	100-125	-	140	Loudon et al., 1985
Macropus eugenii	0.60; 0.52	0.28	90-120	180	140-180	180	Setchell, 1974; Randall et al., 1984
Placentalia							
Mesocricetus auratus	1.81	0.76	16-18	9-10	10	18	Hissa, 1968
Lemmus lemmus	1.80	-	8-10	7-8	1	8	Hissa, 1968
Rattus rattus	2.94-3.30 (10 days)	1.91 (30 days)	12	14-16	1	15-16	Taylor,1960; Gerrish et al., 1998
Microtus oeconomus	3.97	3.15	9	10	11-13	18-30	Ru-Yung and Jinxiang, 1987
Mus musculus	1.76	1.18-1.67	-	15	1	20	Fitzgerald, 1953; Cassin, 1963
Ondatra zibethicus	2.45	1.49	-	-	10-11	14-15	MacArthur and Humphries, 1999
Clethrinonomys rutilus	-	2.75	-	7-10	-	17	Morrison et al., 1954
Cavia porcellus	0.98-1.13	0.72	-	0	1	1	Adamsons et al., 1969

Species	Neonatal mass specific standard metabolic rate (ml O_2*g^{-1}*hr^{-1})	Adult mass specific standard metabolic rate (ml O_2*g^{-1}*hr^{-1})	Attaining of adult metabolic rate (day)	Covering with pelage (day)	Onset of thermoregulation (day)	Full thermogenic capacity (day)	Reference
Crocidura russula	3.00	2.45	-	-	-	14	Nagel, 1989
Suncus murinus	4.32	1.84	8	-	First week	8	Dryden et al., 1974
Erinaceus europaeus	-	0.40	-	20	-	31	Müller, 1972
Dasypus novemcinctus	1.93	0.20	8	-	-	-	Bagatto et al., 2000;
Felis domestica	2.24-2.32	0.71	10	12	-	16	Wollburg, 1957; Hull, 1965;Frappell et al., 1991
Canis familaris	0.9	0.3-0.5	-	0	1	21	Crighton, 1974
Oryctolagus cunniculus	1.5	0.68	10	8	1	10	Hull, 1965
Lepus europaeus	-	0.80	-	0	1	7	Hackländer et al., 2002
Ovis aries	0.62	0.40	At day of birth	0	-	-	Barcroft, 1948; Fahey and Lister, 1989
Bos taurus	0.41	0.17	-	0	-	-	Reeves and Leathers, 1964
Phoca vitulina	0.74	0.47	-	0	-	At birth	Iversen and Krog, 1973; Miller et al., 1976
Pagophilus groenlandicus	0.9	0.20	-	0	-	At birth	Iversen and Krog, 1973

(Bryant and Hails, 1975; Withers and Jarvis, 1980; Vinter et al., 1982). Furthermore, under natural conditions, females may build better-insulating nests as ambient temperatures fall (Barnett, 1956; Lynch, 1974), thus assisting thermoregulation by their young. Precocially born placentals have a pelage, can stand and walk, lack an insulating nest or warming by the mother, and are able to thermoregulate at birth. Examples for this precocial thermoregulation are newborn *Cavia porcellus* (Brück and Wünneberg, 1966), *Lepus europaeus* (Hackländer et al., 2002), *Rangifer tarandus* (Hart et al., 1961), and *Sus domestica* (Dividich and Noblet, 1981). The altricial-precocial contrast with regard to the thermoregulatory development can be found also in birds (Nichelmann et al., 1998, 1999, 2001; Nichelmann and Tzschentke, 2002). In small altricial placental newborns non-shivering thermogenesis is widespread (Sundin and Cannon, 1980; Sundin et al., 1981), whereas in larger precocial newborns non-shivering and shivering thermogenesis can be found (Brück and Wünneberg, 1966). Brown

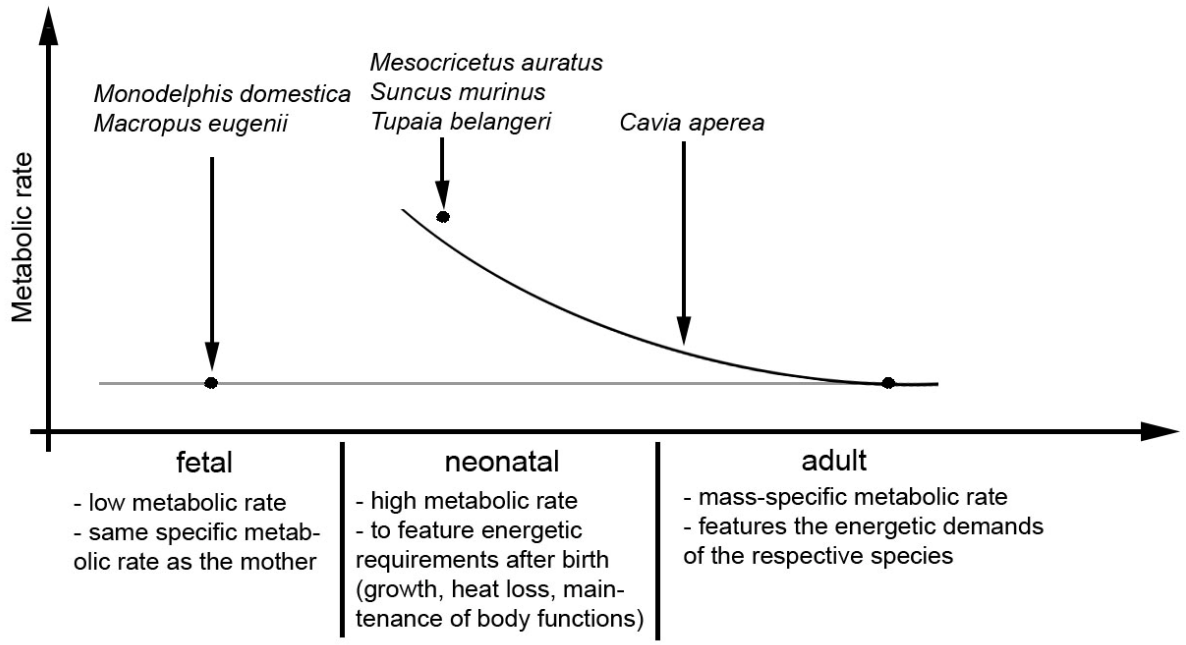

Fig. 110: Summary diagram of respiratory capacity in the six mammalian species examined. Arrows show the developmental degree at birth for each species.The newborn marsupials have low metabolic rates which correspond to the fetal metabolism. Neonatal placentals increase the respiratory capacity after birth and have high metabolic rates. The precocial guinea pig has approximately an adult metabolic rate at birth.

adipose tissue is present in a variety of newborn placentals and is concerned with non-shivering thermogenesis (Smith and Horowitz, 1969; Gemmell et al., 1972; Alexander et al., 1974), thereby contributing substantially to cold resistance (Hull and Segall, 1965; Alexander, 1970).

Summarising the metabolic and thermoregulatory development of marsupials and placentals, it becomes clear, that marsupials are generally born with a low metabolic rate and without thermogenic capacity. They persist on this low “fetal” metabolic level for long time and attain adult metabolic rates and thermoregulatory abilities late in the postnatal development. In contrast, placentals have high metabolic rates (higher than adults) at birth and show even in altricial condition first thermoregulatory response. They reach adult metabolism within the first week of life and attain full thermogenic capacity early in the postnatal development. Precocially born placentals are fully mature in terms of metabolic rate and thermoregulation. The results are summarised in figure 110.

4.4 Morphotype reconstruction of the neonates of Marsupialia and Placentalia

For the reconstruction of the evolution of reproductive strategies in Marsupialia and Placentalia, the morphotypes, which are the sum of characters present in the last common

ancestors (Hennig, 1950; Königsmann, 1975), of Theria, Marsupialia, Placentalia and internal groups must be reconstructed (Zeller and Freyer, 2001). The morphotype reconstruction with regard to placentation and early ontogeny of the Marsupialia is already finished (Zeller and Freyer, 2001) and the morphotype reconstruction with regard to placentation of the Placentalia is currently subject of investigation. What still is missing is the morphotype reconstruction for the neonates of Marsupialia and Placentalia, which can be understood as a result of the placentation. This study is a contribution to the reconstruction of the neonatal morphotypes of recent marsupials and placentals by providing current findings, a character polarisation and a phylogenetic interpretation. The characters examined belong to the external morphology (character 1-4), to the lung development (character 5-9), and to the metabolic development (character 10-12) of the neonates. The marsupial morphotype reconstruction is based on the systematics as proposed by Luckett (1994) (see Fig. 1) and the placental morphotype reconstruction uses the systematics as proposed by Springer et al. (2003) (see Fig. 2).

The data presented in this study support most of the previous examinations on the external characters, the lung development and the metabolic development of marsupial and placental neonates. In marsupials, the complete metabolic development of *Monodelphis domestica* is described for the first time. In placentals, the lung development of *Suncus murinus* and *Tupaia belangeri*, and the metabolic development of *Tupaia belangeri* were investigated for the first time.

4.4.1 Marsupialia

Table 13 and figure 111 summarise characters that are concluded to be present in the marsupial morphotype and those that are derived, based on the character distribution within marsupials. The neonate in the marsupial morphotype is born with a low birth weight, less than 1 g, is naked, has closed eyes, pronounced forelimbs and embryonic hindlimbs. The neonatal lung is at the air sac stage of lung development with large terminal air sacs, which are separated by thick double capillary septa. Furthermore, a poor developed bronchial system with short lobar bronchi, which open directly into the large air sacs, and a late formation of alveoli are characteristic for the marsupial neonate. The neonate in the marsupial morphotype has a low metabolic rate and attains the adult metabolic rate and thermoregulatory capacity late in the postnatal development. This pattern of the marsupial neonate is also the morphotype of Ameridelphia and Australidelphia respectively. From the marsupials investigated in this study, *Monodelphis domestica* represents the marsupial morphotype best of all. Whereas the Ameridelphia, in particular the Didelphidae remained originally and show all characters of the marsupial morphotype, the Australidelphia are

Tab. 13: Characters of the marsupial morphotype (0) and apomorphic characters (1).

Character no.	Character state 0 (morphotype)	Taxon	Character state 1 (apomorphy)	Taxon	Character not applicable [?]	Taxon
1	Low birth weight (< 1g)	Didelphidae; Peramelidae; Vombatidae; Phalangeridae; Potoroidae; Macropodidae; Burramyridae; Pseudocheiridae; Petauridae	Extreme low birth weight (< 0.018 g)	Dasyuridae; Tarsipedidae		
2	Closed eyes at birth	Didelphidae; Dasyuridae; Peramelidae; Phalangeridae; Potoroidae; Macropodidae; Pseudocheiridae; Petauridae; Acrobatidae; Tarsipedidae				
3	Lack of pelage at birth	Didelphidae; Dasyuridae; Peramelidae; Phalangeridae; Potoroidae; Macropodidae; Pseudocheiridae; Petauridae; Tarsipedidae				
4	Pronounced forelimbs and embryonic hindlimbs at birth	Didelphidae; Dasyuridae; Peramelidae; Phalangeridae; Potoroidae; Macropodidae; Pseudocheiridae; Petauridae; Acrobatidae; Tarsipedidae				
5	Lung at air sac stage at birth	Didelphidae; Dasyuridae; Peramelidae; Phalangeridae; Macropodidae				
6	Large terminal air sacs at birth	Didelphidae; Peramelidae; Phalangeridae; Macropodidae	Two air sacs with septal crest	Dasyuridae		
7	Thick double capillary septum at birth	Didelphidae; Peramelidae; Phalangeridae; Macropodidae			No double capillary septum at birth	Dasyuridae
8	Late postnatal formation of alveoli	Didelphidae; Peramelidae; Phalangeridae; Macropodidae				
9	few developed bronchial tree at birth	Didelphidae; Peramelidae; Phalangeridae	More developed bronchial tree at birth	Macropodidae	No bronchial tree at birth	Dasyuridae
10	Low metabolic rate at birth	Didelphidae; Macropodidae				
11	Late attainment of adult metabolism	Didelphidae; Dasyuridae; Macropodidae				
12	Late attainment of thermoregulation	Didelphidae; Dasyuridae; Phalangeridae; Potoroidae; Macropodidae				

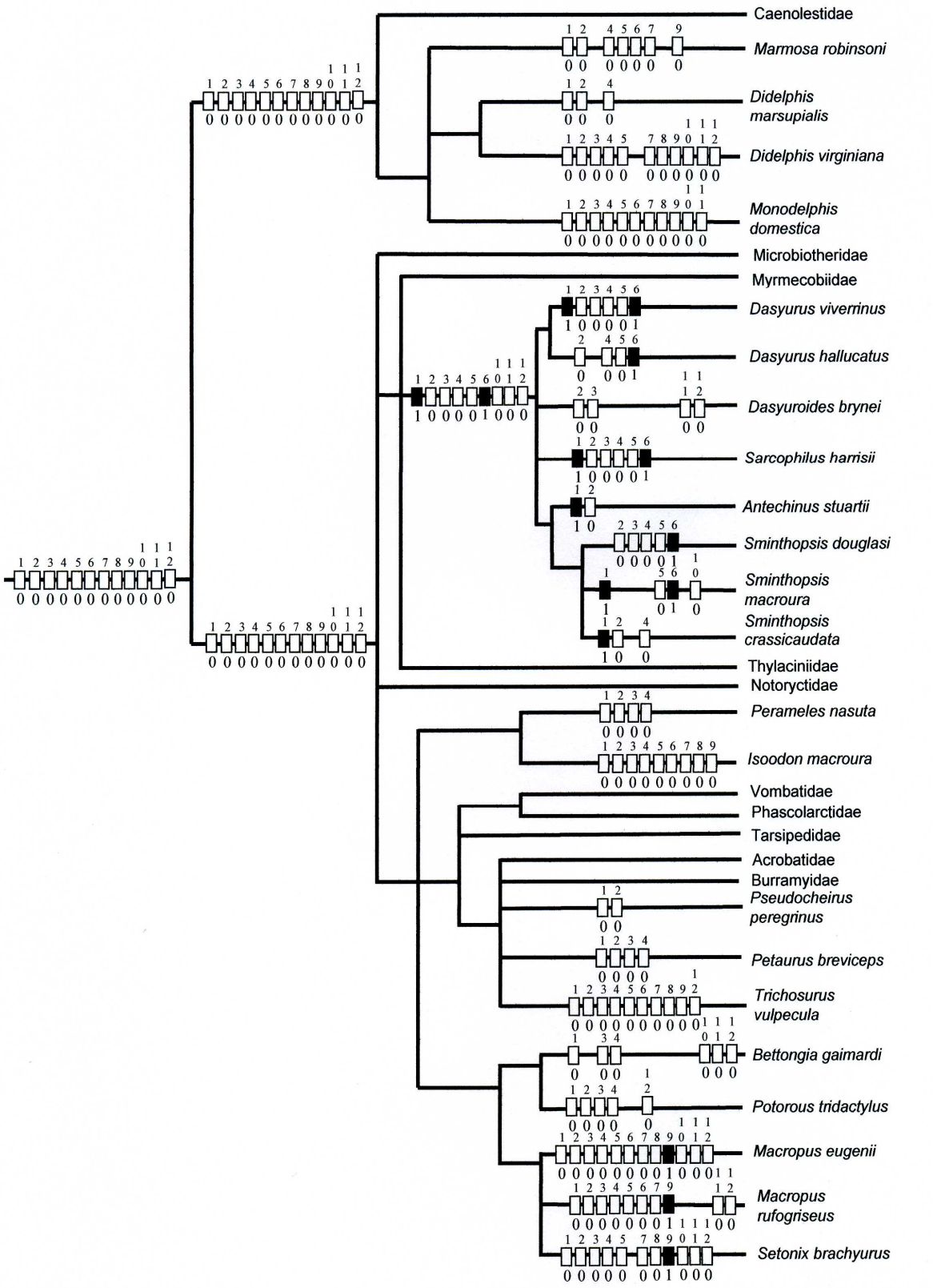

Fig. 111: Reconstruction of the neonate in the marsupial morphotype using parsimony as described by Cunningham et al. (1998). For character coding see Table 13. Numbers above rectangle are character numbers; the numbers below rectangle are the character states.

derived in part more strongly. Apomorphic characters can be found in the Dasyuridae and in the Macropodidae. The neonate of the Dasyuridae is born with an extreme low birth weight (0.005-0.018 g) and is structurally more immature than the marsupial morphotype. The newborn lung comprises two air sacs with only few septal crests protruding into the air sac lumen. In comparison with the marsupial morphotype, the dasyurids are derived towards a more immature born neonate, which is characterised by the previously described developmental degree G1. This point of view is also supported by Asher et al. (2004). Within the neonate of the macropodides, a more developed bronchial tree with long lobar bronchi can be found. Besides the large terminal air sacs also smaller air sacs are present at birth. In comparison with the marsupial morphotype, the macropodides are derived towards a more mature born neonate, which is characterised by the previously described developmental degree G3.

4.4.2 Placentalia

The characters that are concluded to be present in the placental morphotype and those that are derived, based on the character distribution within placentals, are summarised in table 14 and figure 112. The neonate in the placental morphotype is born at a relative high birth weight, more than 1 g, is naked, has closed eyes, and well developed extremities. The neonatal lung is at the air sac stage with small terminal air sacs which are separated by thin double capillary septa. The bronchial system of the newborn lung is well developed and extends with many dichotomies deep into the periphery of the lung, but is still incomplete and lacks respiratory bronchi. In the placental morphotype, the formation of alveoli begins short after birth. The neonate in the placental morphotype has a high metabolic rate and attains the adult metabolic rate and thermoregulatory capacity early in the postnatal development. This morphotype of the placental neonate is present mainly in the Soricidae and Muridae, which remained originally and show all characters of the placental morphotype. They are referred as typical altricial species. From the placentals investigated in this study, *Suncus murinus* resembles the placental morphotype most closely. Due to the enormous radiation of the placentals after the K/T-boundary, many orders of the Placentalia a more derived than that of the Marsupialia. Some taxa, like tupaias and rabbits, have the appearance of the placental morphotype (closed eyes, lack of pelage at birth), but are derived with regard to the lung structure. They are already at the alveolar stage of lung development, and have alveoli and single capillary septa already at birth. The neonates of cats and dogs are still developed a little more, with closed eyes, but with pelage at birth. They are also at the alveolar stage of lung development at birth and have a complete developed bronchial tree with respiratory bronchioles. However, the most derived neonates can be found in the cetartiodactyles, the pinnipeds, the perissodactyles, the hystricognath rodents, the primates, the macroscelides,

Tab. 14: Characters of the placental morphotype (0) and apomorphic characters (1).

Character no.	Character state 0 (morphotype)	Taxon	Character state 1 (apomorphy)	Taxon	Character not applicable [?]	Taxon
1	High birth weight (> 1 g; except some small Soricidae, which have smaller newborns)	Afrocoricida; Macroscelidea; Tubulidenta; Sirenia; Hyracoidea; Proboscidea; Xenarthra; Primates; Scandentia; Lagomorpha; Rodentia; Eulipothyphla; Chiroptera; Pholidota; Carnivora; Perissodactyla; Cetartiodactyla				
2	Closed eyes at birth	Afrocoricida; Tubulidenta; Dasypodidae; Tupaiidae; *Oryctolagus;* Ochotonidae; Sciurognathi; Eulipothyphla; Fissipedia;	Open eyes at birth	Macroscelidea; Sirenia; Hyracoidea; Proboscidea; Bradypodidae; Primates; *Lepus;* Hystricognathi; Chiroptera; Pholidota; Pinnipedia; Perissodactyla; Cetartiodactyla		
3	Lack of pelage at birth	Scandentia; Ochotonidae; Muridae; Eulipothyphla; Ursidae	Pelage at birth	Macroscelidea; Hyracoidea; Bradypodidae; Primates; *Lepus;* Hystricognathi; Canidae; Felidae; Pinnipedia; Perissodactyla; Artiodactyla	Pelage not present	Sirenia; Proboscidea; Dasypodidae; Pholidota; Cetacea
4	Well developed extremities at birth	Afrocoricida; Macroscelidea; Tubulidenta; Sirenia; Hyracoidea; Proboscidea; Xenarthra; Primates; Scandentia; Lagomorpha; Rodentia; Eulipothyphla; Chiroptera; Pholidota; Carnivora; Perissodactyla; Cetartiodactyla				
5	Lung at air sac stage at birth	Soricidae; Muridae	Lung at alveolar stage at birth	Macroscelidae; Caviidae; Leporidae; Felidae; Canidae; Suidae; Bovidae; Cercopithecidae; Pongidae; Tupaiidae		

Character no.	Character state 0 (morphotype)	Taxon	Character state 1 (apomorphy)	Taxon	Character not applicable [?]	Taxon
6	Small terminal air sacs at birth	Soricidae; Muridae	Small alveoli at birth	Macroscelidae; Caviidae; Leporidae; Felidae; Canidae; Suidae; Bovidae; Tupaiidae; Cercopithecidae; Pongidae		
7	Thin double capillary septum at birth	Soricidae; Muridae	Single capillary septum at birth	Macroscelidae; Caviidae; Leporidae; Felidae; Canidae; Suidae; Bovidae; Tupaiidae; Cercopithecidae; Pongidae		
8	Early postnatal formation of alveoli	Soricidae; Muridae	Alveoli already present at birth	Macroscelidae; Caviidae; Leporidae; Felidae; Canidae; Suidae; Bovidae; Tupaiidae; Cercopithecidae; Pongidae		
9	Well developed bronchial tree at birth	Soricidae; Muridae; Tupaiidae	Complete bronchial tree at birth	Macroscelidae; Caviidae; Leporidae; Felidae; Canidae; Suidae; Bovidae; Cercopithecidae; Pongidae		
10	High metabolic rate at birth	Soricidae; Muridae; Cavidae; Dasypodidae; Felidae; Canidae; Tupaiidae; Leporidae; Bovidae; Phocidae				
11	Fast attainment of adult metabolism	Soricidae; Muridae; Cavidae; Dasypodidae; Tupaiidae; Felidae; Leporidae; Bovidae				
12	Fast attainment of thermoregulation	Soricidae; Erinaceidae; Muridae; Cavidae; Felidae; Canidae; Leporidae; Phocidae				

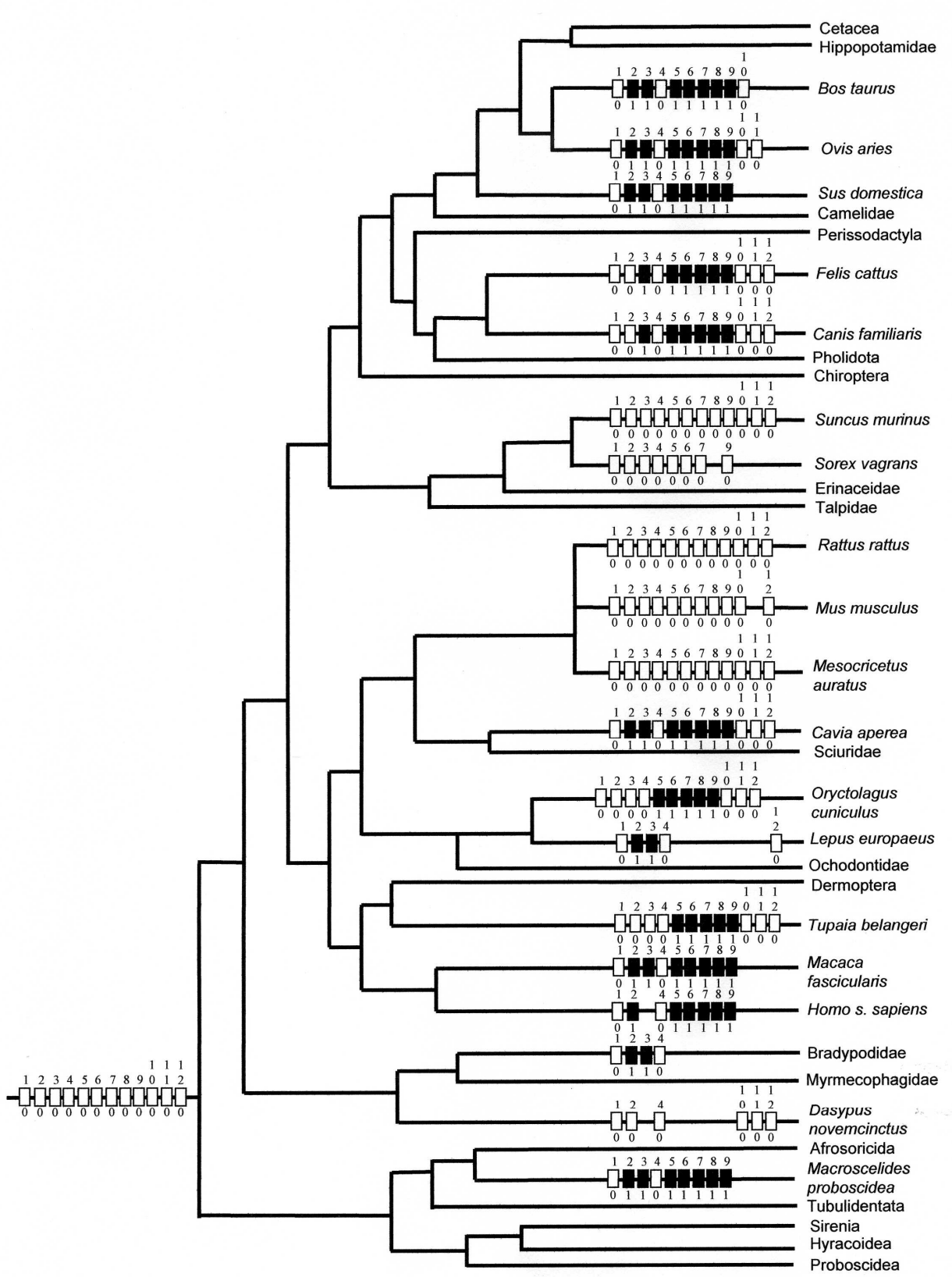

Fig. 112: Reconstruction of the neonate in the placental morphotype using parsimony as described by Cunningham et al. (1998). Species are choosen, which have the most complete data set of the characters investigated. For character coding see Table 14. Numbers above rectangle are character numbers; the numbers below rectangle are the character states.

and the paeungulates. They all have highly precocious neonates, with open eyes and pelage at birth. The lungs of newborn Bovidae, Cavidae, Cercopithecidae, Pongidae, and Macroscelidae are already at the alveolar stage, with small alveoli, single capillary septa, and a complete developed bronchial tree with respiratory bronchioles. The metabolic development is very similar in all placental neonates and corresponds to the placental morphotype.

4.5 Concluding discussion

The morphotype reconstruction of the marsupial and placental neonates reveals some striking differences in their structural prerequisites. The newborn marsupial is characterised by highly immature lungs with large terminal air sacs, which provide only a small surface area for the gas exchange. The lungs are adequate for the normal requirements in the most newborn marsupials. In the extreme small dasyurids, with the most immature lungs of all marsupial neonates, the respiratory requirements are additionally supplemented by gas exchange through the skin. The immature structure of the newborn marsupial lung enables only a low metabolic rate at birth. A rise of the metabolic rate and increased heat production in response to lowered ambient temperature is not possible in marsupials for a long time after birth. At the one hand the structural prerequisites for high metabolic rates are missing in young marsupials, but on the other hand the low metabolic rate could be an adaptation of the young to save energy by getting all its energy needs from the mother, just as a placental fetus. Baudinette et al. (1988) suggests that this pattern of slow postnatal metabolic development may be an adaptation to a reduced need for heat production in the pouch. However, because this pattern is present also in pouch less marsupials, it is more probably that it is an original character of the marsupial morphotype. In addition, the small size of the marsupial neonate, the lack of pelage, and the absence of a protecting pouch in the early marsupials predisposes it to hypothermia and potential desiccation.

In contrast to the marsupials, the neonate in the placental morphotype has more developed lungs with numerous small terminal air sacs, which provide a relatively large surface area for the gas exchange. With that advanced structural prerequisite, the placental neonate can realise a high metabolic rate at birth and give a first thermoregulatory response to lowered ambient temperatures short after birth. With consideration of the climate cooling after the K/T-event, the general high degree of immaturity, the immature lungs, and the slow metabolic and thermoregulatory development of the marsupial offspring, may be definitely a limiting factor. Hughes and Hall (1988) describe the neonate as the most vulnerable stage in the mammalian life cycle. In this respect, the marsupial offspring can be considered as a

prolonged stage of the neonatal status (naked, without thermoregulation) and for this reason vulnerable for a long time during the postnatal development. In contrast, the placental offspring develops rapid (fast furring and thermoregulation) and leaves the vulnerable stage, characteristic for neonates, early in the postnatal development. Taking into consideration the differences in the developmental degree at birth and in the developmental rate, a climate cooling after the K/T-event may have affected the marsupial offspring much more than the placental offspring.

One of the most important inventions of marsupials is the pouch, which probably evolved during the Miocene and is associated with the evolution of large size and the carriage of young for a long period (Russell, 1982). With the dislocation of the postnatal development into the protective and constant warm environment of the pouch, the marsupials solved the problem of the vulnerability, hypothermia and desiccation of their offspring.

The difference in the developmental degree of the neonates is only one aspect of the reproductive strategies of marsupials and placentals, which may have led to the different evolutionary development in these both groups. Several other aspects of the reproductive biology, such as the fast reproductive cycles, the large litter sizes, and the rapid development of the offspring with an early onset of sexual maturity in Placentalia, were maybe advantageous in the fast colonisation of the empty landscape after the K/T-event. In South America, where placentals and marsupials occured together, the marsupial did not undergo such an extensive adaptive radiation as in Australia, where the marsupials became physically isolated from the placentals over 100 million years ago. One explanation could be that placental mammals were apparently more successful and took over most of the herbivorous ways of life. Another possibility may be that larger species of marsupials take longer to evolve and are more vulnerable to extinction due to longer interbirth intervals. Nethertheless marsupials were, and still are, successful as insectivores, carnivores, and small herbivores. Two of the most successful marsupial families are the Didelphidae in South America and the Macropodidae in Australia. The didelphids have high litter sizes and relative fast reproductive cycles in comparison with other marsupials. They are generalists and very successful, due to their commensal life style and their opportunistic nutrition. The didelphids are widespread through South America and it is the only mammal currently still extending its home range in North America. The macropodids have evolved another reproductive strategy, with a single young once the year. They are well adapted to the desert conditions in Australia, where most of the placental mammals can not survive. However, the most marsupials of the endemic Australian fauna are highly endangered due to the introduced species syndrome, where resident animals are not adapted to competing with new species.

Altogether, the reproductive strategy of the placental mammals seems to be more successful than that of the marsupials. Placental mammals have radiated to a wide variety of forms and

are adapted to nearly all habitats of the world, including the sea and the air. Derived from the relative immature altricial neonates, the placentals have evolved highly precocial newborns, which are the prerequisite for certain life strategies, such as living in the marine environment or in open grasslands.

The characters examined concerning general development, lung development and metabolic development and their fusion into a morphotype reconstruction of marsupial and placental neonates is one step toward an evolutionary holistic scenario that explains mammalian evolution around the Cretaceous-Tertiary boundary.

5 Summary

This project deals with the possible reasons for the evolutionary differentiation between marsupial and placental mammals after the K/T-event. One explanation could be their different reproductive patterns. Metatherians bear virtually embryonic young after a brief gestation period. In contrast, many eutherians bear anatomically advanced, highly precocious young after a relatively long gestation period. A stable metabolism and thermoregulatory abilities of the young are considered to offer a large adaptive advantage in a changing environment, how it is presumed for the K/T-boundary. Therefore this study determines the developmental stage and the respiratory efficiency of the lungs of marsupial and placental young. Histological, ultrastructural and calorimetric investigations were carried out in an integrated study and from the results morphotype reconstructions of the marsupial and placental neonates were carried out. As representatives for altricial Placentalia, the golden hamster (*Mesocricetus auratus*), the musk shrew (*Suncus murinus*), and the Belanger's tree shrew (*Tupaia belangeri*) were examined. Furthermore the Guinea pig (*Cavia aperea*) and the short-eared elephant shrew (*Macroscelides proboscideus*) as typical precocial Placentalia were included. The Marsupialia were represented by the gray short-tailed opossum (*Monodelphis domestica*) and the tammar wallaby (*Macropus eugenii*). The results confirm clear differences in the developmental degree of the neonates and the postnatal development between marsupial and placental mammals. The newborn lungs of the altricially born placentals *M. auratus* and *S. murinus* are at the late terminal air sac stage with numerous small air sacs of 50 - 80 µm in diameter. Alveoli are formed shortly after birth at the age of 2 days in *M. auratus* and at the age of 4 days in *S. murinus*. In *T. belangeri* and in the precocially born *C. aperea* and *M. proboscideus* alveoli are already present at birth. In contrast, the lungs of the newborn marsupials *M. domestica* and *M. eugenii* are at the early terminal air sac stage with few large air sacs of 300 – 400 µm in diameter. The postnatal lung development proceeds very slowly in marsupials and alveoli are not present before the age

of 28 days *in M. domestica* and 65 days in *M. eugenii*. The metabolic investigations demonstrate that Marsupialia have a low metabolism at birth and achieve the adult metabolism late in the postnatal development. All Placentalia examined showed the same pattern of oxygen consumption. Corresponding to their advanced differentiation of the lungs they also exhibit high metabolic abilities at birth and reach the adult metabolism during the first week of life. During this critical period placental young have a higher resistance against certain environmental stresses than marsupial young and this could mean an evolutionary advantage of the placental "reproductive strategy" under suboptimal climatic conditions.

6 Zusammenfassung

Die Dissertation beschäftigt sich mit den möglichen Gründen für die divergente evolutionäre Entwicklung von Beuteltieren und plazentalen Säugern nach der Kreide/Tertiär-Grenze. Eine Erklärung könnten ihre unterschiedlichen Reproduktionsstrategien sein. Während die Beuteltiere sehr embryonale Jungtiere nach einer kurzen Tragzeit gebären, bringen Plazentalier deutlich weiter entwickelte Jungtiere nach einer relativ langen Tragzeit zur Welt. Die Aufrechterhaltung eines stabilen Metabolismus und thermoregulatorische Fähigkeiten der Jungtiere bieten einen großen Vorteil für die Anpassungsfähigkeit an ungünstige Umweltbedingungen, wie sie für die K/T-Grenze vermutet werden. Aus diesem Grund stehen in dieser Studie insbesondere der strukturelle Entwicklungsgrad der Lunge und die metabolischen Fähigkeiten von neonaten Marsupialia und Plazentalia im Mittelpunkt des Interesses. Histologische, ultrastrukturelle und kalorimetrische Untersuchungen erfolgten in einer integrativen Studie. Basierend auf den Ergebnissen wurde eine Grundplanrekonstruktionen der Neonaten von Marsupialia und Plazentalia durchgeführt. Als Vertreter für nesthockende Plazentalia wurde der Goldhamster (*Mesocricetus auratus*), die Moschusspitzmaus (*Suncus murinus*) und das Belangeri Spitzhörnchen (*Tupaia belangeri*) untersucht. Des Weiteren wurden das Wildmeerschweinchen (*Cavia aperea*) und der Kurzohr-Rüsselspringer (*Macroscelides proboscideus*) als typischer Vertreter der nestflüchtenden Plazentalia in die Untersuchung mit einbezogen. Die Marsupialia sind durch die Hausspitzmaus-Beutelratte (*Monodelphis domestica*) und das Tammar-Wallaby (*Macropus eugenii*) vertreten.

Die Ergebnisse bestätigen die starken Unterschiede im Entwicklungsgrad der Neonaten und in der postnatalen Entwicklung zwischen Marsupialia und Plazentalia. Die neonatalen Lungen der als Nesthocker geborenen *M. auratus* und *S. murinus* befinden sich im späten "terminal air sac"-Stadium und weisen viele kleine Atemkammern von 50-80 µm Durchmesser auf. Die Alveolenbildung erfolgt bereits kurz nach der Geburt, im Alter von zwei

Tagen bei *M. auratus* und im Alter von vier Tagen bei *S. murinus*. Bei *T. belangeri* und dem als Nestflüchter geborenen *C. aperea* sind Alveolen bereits zum Zeitpunkt der Geburt vorhanden. Im Gegensatz dazu, befinden sich die Lungen der neonaten Beuteltiere M. domestica und M. eugenii im frühen “terminal air sac”-Stadium mit nur wenigen großen Atemkammern von 300-400 µm im Durchmesser. Die postnatale Lungenentwicklung erfolgt sehr langsam bei den Marsupialia und die Alveolenbildung findet nicht vor 28 Tagen bei *M. domestica* und 65 Tagen bei *M. eugenii* statt. Die Metabolismusmessungen ergaben, daß Marsupialia mit einer niedrigen Metabolismusrate geboren werden und den Adultmetabolismus erst spät in der postnatalen Entwicklung erreichen. Die untersuchten Plazentalia zeigten alle das gleiche Muster im Sauerstoffverbrauch. Einhergehend mit der weit entwickelten Lungenstruktur weisen die Plazentalia hohe Metabolismusraten zur Geburt auf und erreichen den Adultmetabolismus innerhalb der ersten Lebenswoche.

Während dieses kritischen Lebensabschnittes haben die Jungtiere der Plazentalia eine höhere Widerstandskraft gegen Umweltschwankungen als die Jungtiere der Marsupialia, was als ein evolutiver Vorteil der Reproduktionsstrategie der Plazentalia unter ungünstigen Klimabedingungen interpretiert werden kann.

References

Adamson, I.Y.R. and Bowden, D.H. 1973. The intracellular site of surfactant synthesis: autoradiographic studies on murine and avian lung explants. – Exp. Molec. Pathol. 18: 112-124.

Adamson, I.Y.R. and Bowden, D.H. 1975. Derivation of type 1 epithelium from type 2 cells in the developing rat lung. – Lab. Inv. 32 *(6)*: 736-745.

Adamsons, K., Blumberg, E. and Joelsson, I. 1969. The effect of ambient temperature upon post-natal changes in oxygen consumption of the Guinea-Pig. – J. Physiol. 202: 261-269.

Alcorn, D.G., Adamson, T.M., Maloney, J.E. and Robinson, PM. 1981. A morphologic and morphometric analysis of fetal lung development in the sheep. – Anat. Rec. 201: 655-667.

Alexander, G. 1970. Thermogenesis in young lambs. – In: Phillipson, A.T. (ed.). Physiology of Digestion and Metabolism in the Ruminant. Oriel Press. U.K.

Alexander, G., Bennett, J.W. and Gemmell, R.T. 1974. Brown adipose tissue in the newborn calf. – J. Physiol. 244: 223-234.

Albignac, R. 1973. Mammiferes carnivores. Faune de Madagascar. Vol.36. Orstom. Paris.

Alroy, J. 1999. The fossil record of North American mammals: evidence for a Palaeocene evolutionary radiation. – Syst. Biol. 48: 107-118.

Archer, M., Murray, P., Hand, S. and Godthelp, H. 1993. Reconsideration of monotreme relationships based on the skull and dentition of the Miocene Obdurodon dicksoni. – In: Szalay, F.S., Novacek, M.J. and McKenna, M.C. (eds.). Mammal Phylogeny, vol.1. Mesozoic Differentiation, Multituberculates, Monotremes, Early Therians, and Marsupials. Springer-Verlag. New York.

Archibald, J.D. 1982. A study of Mammalia and geology across the Cretaceous-Tertiary boundary in Garfield County, Montana. – Geol. Sciences. 122: 1-286.

Archibald, J.D. 1996a. Fossil evidence for a late Cretaceous origin of “hoofed” mammals. – Science. 272: 1150-1153.

Archibald, J.D. 1996b. Dinosaur extinction and the end of an era: what the fossils say. Columbia University Press. New York.

Archibald, J.D. and Bryant, L.J. 1991. Differential Cretaceous/Tertiary extinctions of nonmarine vertebrates: evidence from northeastern Montana. – Geol. Soc. Am. Spec. Pap. 247: 549-562.

Ardito, G. 1975. Checklist of the data on the gestation length of primates. Instituto di Antropologia. Torino.

Arlton, A.V. 1936. An ecological study of the mole. – J. Mammal. 17: 349-371.

Arnason, U., Adegoke, J.A., Bodin, K., Born, E.W., Wsa, Y.B., Gullberg, A., Nilsson, M., Short, R.V., Xu, X. and Janke, A. 2002. Mammalian mitogenomic relationships and the root of the eutherian tree. - Proc. Natl. Acad. Sci. 99 *(12)*: 8151-8156.

Asdell, S.A. 1964. Patterns of mammalian reproduction. 2th ed. Cornell University Press. Ithaca.

Asher, R.J., Horowitz, I. and Sanchez-Villagra, M.R. 2004. First combined cladistic analysis of marsupial mammal interrelationships. – Molec. Phylogen. Evol.. 33: 240-250.

Askin, R.A. 1992. Late Cretaceous-Early Tertiary Antarctic outcrop evidence for past vegetation and climates. – In: Kennett, J.P., Warnke, D.A (eds.). The Antarctic Palaeoenvironment: A perspective on Clobal change, Part 1. Arctic Research Series 56. American Geophysical Union.

Bagatto, B., Crossley II, D.A. and Burggren, W.W. 2000. Physiological variability in neonatal armadillo quadruplets: Within- and between-litter differences. – J. Exp. Biol. 203: 1733-1740.

Banks, D.R. 1978. Hand-rearing a musk-ox, *Ovibos moschatus*, at Calgary Zoo. – Int. Zoo Yearb. 18: 213-215.

Barnes, R.D. 1977. The special anatomy of *Marmosa robinsoni*. – In: Hunsaker, D (ed.). The biology of marsupials. Academic Press. New York. San Francisco. London.

Barnett, S.A. 1956. Endothermy and ectothermy in mice at -3°C. – J. Exp. Biol. 33: 124-133.

Barrette, C. 1975. Social behaviour of muntjac. Ph.D. thesis. Department of Biology. University of Calgary.

Baudinette, R.V., Gannon, B.J., Ryall, R.G. and Frappell, P.B. 1988a. Changes in metabolic rates and blood respiratory characteristics during pouch development of a marsupial, *Macropus eugenii*. – Resp. Physiol. 72: 219-228.

Baudinette, R.V., Runciman, S.I.C. Frappell, P.F. and Gannon, B.J. 1988b. Development of the marsupial cardiorespiratory system. - In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

Barcroft, J. 1948. Researches on prenatal life. Charles C. Thomas Co. Springfield, Illinois.

Baverstock, P.R., Krieg, M. and Birrell, J. 1990. Evolutionary relationships of Australian marsupials as assessed by Albumin Immunology. – Aust. J. Zool. 37: 273-287.

Bedford, J.M., Mori, T. and Oda, S. 1997. Ovulation induction and gamete transport in the female tract of the musk shrew, *Suncus murinus*. – J. Reprod. Fert. 110: 115-125.

Bekoff, M. and Jamieson, R. 1975. Physical development in coyotes (*Canis latrans*) with a comparison to other canids. – J. Mammal. 56: 685-692.

Bensch, K., Schaefer, K. and Avery, M.E. 1964. Granular pneumocytes: Electron microscopic evidence of their exocrinic function. – Science. 145: 1318-1319.

Benton, M.J. 1991. The rise of the mammals. Crescent books. New York.

Bickel, C.L., Murdock, G.K. and Smith, M.L. 1976. Hand-rearing a giant anteater at Denver Zoo. – Int. Zoo Yearb. 16: 195-198.

Birkenholz, D.E. and Wirtz, W.O. III. 1965. Laboratory observations on the vesper rat. – J. Mammal. 46: 181-189.

Blackburn, D.G. 1992. Convergent evolution of viviparity, maltrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. – Am. Zool. 32: 313-321.

Blackburn, D.G. 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. – J. Theor. Biol. 174: 199-216.

Blair, F.W. 1941. Observations on the life history of *Baiomys taylori* subater. – J. Mamm. 22: 378-383.

Bland, D.K. and Holland, R.A.B. 1977. Oxygen affinity and 2,3-Diphosphoglycerate in blood of Australian marsupials of differing body size. – Respir. Physiol. 31: 279-290.

Block, J.A. 1974. Hand-rearing seven-banded armadillos, *Dasypus septemcinctus*, at the National Zoological Park, Washington. – Int. Zoo Yearb. 14: 210-214.

Bokma, F. 2004. Evidence against universal metabolic allometry. – Funct. Ecol. 18: 184-187.

Bourliere, F., Petter-Rousseaux, A. and Petter, J.J. 1961. Regular breeding in captivity of the lesser mouse lemur (*Microcebus murinus*). – Int. Zoo Yearb. 3: 24-25.

Boyden, E.A. and Tompsett, D.H. 1961. The postnatal growth of the lung in the dog. – Acta Anat. 47: 185-215.

Bradt, G.W. 1938. A study of beaver in Michigan. – J. Mammal. 19: 139-162.

Brady, C.A. and Ditton, M.K. 1979. Management and breeding of maned wolves, *Chrysocyon brachyurus*, at the National Zoological Park, Washington. – Int. Zoo Yearb. 19: 171-176.

Bremer, J.L. 1904. On the lung of the opossum. – Amer. J. Anat. 3: 67-73.

Broadbrooks, H.E. 1958. Life history and ecology of the chipmunk, *Eutamias amoenus*, in eastern Washington. – Misc. Publ. Mus. Zool. Univ. Mich. No. 103.

Brody, J.S. and Vaccaro, C. 1979. Posnatal formation of alveoli: interstitial events and physiologic consequences. – Federation Proc. 38 *(2)*: 215-223.

Bronner, G.N. 1992. Notes on the early post-natal development of a giant golden mole *Chrysospalax trevelyani* (Günther, 1875) born in captivity (Mammalia: Insectivora; Chrysochloridae). – Koedoe. 35 *(2)*: 57-58.

Bronson, F.H. 1985. Mammalian Reproduction: An ecological perspective. – Biol. Reprod. 32: 1-26.

Bryant, D.M. and Hails, C.J. 1975. Mechanisms of heat conservation in the litters of mice (*Mus musculus* L.). – Comp. Biochem. Physiol. A. 50: 99-104.

Bryden, M.M. 1972. Growth and development of marine mammals. – In: Harrison, R.J. (ed.). Functional anatomy of marine mammals. Academic Press. London.

Buaboocha, W. and Gemmell, R.T. 1997. Development of lung, kidney and skin in the Brushtail possum, *Trichosurus vulpecula*. - Acta Anat. 159: 15-24.

Bucher, O. und Wartenberg, H. 1989. Cytologie, Histologie und mikroskopische Anatomie des Menschen. 11. Auflage, Verlag Hans Huber, Bern Stuttgart, Toronto.

Buckingham, S. and Avery, M.E. 1962. Time of appearance of lung surfactant in the foetal mouse. – Nature. 193: 688-689.

Burda, H. 1989. Reproductive biology (behavior, breeding, and postnatal development) in subterranean mole-rats, *Cryptomys hottentotus* (Bathyergidae). – Z. Säugetierk. 54: 360-376.

Burnie, D. 2001. Tiere. Dorling Kindersley Verlag. München

Burri, P.H. 1974. The postnatal growth of the rat lung. III. Morphology. – Anat. Record. 180: 77-98.

Burri, P.H. 1985. Development and growth of the human lung.- In: Fishman, A.P. & Fishman A.B. (eds.). Handbook of Physiology. Sect. 3, Vol. 1: 1-46.

Burri, P.H., Dbaly, J. and Weibel, E.R. 1974. The postnatal growth of the rat lung. I. Morphometry. – Anat. Rec. 178: 711-730.

Burri, P.H., Haenni, B., Tschanz, S.A. and Makanya, A.N. 2003. Morphometry and allometry of the postnatal marsupial lung development: an ultrastructural study. – Resp. Physiol. Neurobiol. 138: 309-324.

Butler, P.M. 1972. The problem of insectivore classification. – In: Joysey, K.A. and Kemp, T.S. (eds.) Studies in Vertebrate Evolution. Oliver and Boyd, Edinburgh.

Calvert, S.J., Holland, R.A.B. and Gemmell, R.T. 1994. Respiratory properties of the neonatal blood of the common brushtail possum (*Trichosurus vulpecula*). – Physiol. Zool. 67 *(2)*: 407-417.

Cao, Y., Fujiwara, M., Nikaido, M., Okada, N. and Hasegawa, M. 2000. Interordinal relationships and timescale of eutherian evolution as inferred from mitochondrial genome data. – Gene. 259: 149-158.

Carroll, R.L. 1993. Paläontologie und Evolution der Wirbeltiere. Georg Thieme Verlag. Stuttgart, New York.

Cassin, S. 1963. Critical oxygen tensions in newborn, young, and adult mice. – Am. J. Physiol. 205: 325-330.

Castleman, W.L. and Lay, J.C. 1990. Morphometric and ultrastructural study of postnatal lung growth and development in calves. – Am. J. Vet. Res. 151 *(5)*: 789-795.

Cehun, S. 1994. An examination of peri-partum lung development in the tammar wallaby. Master-thesis., University of Melbourne, Victoria.

Chang, C.H., Lin, J.Y., Lin, L.K. and Yu, J.Y.L. 1999. Annual reproductive patterns of female House shrew, *Suncus murinus*, in Taiwan. - Zoological Science 16: 819-826.

Chapman, D. and Chapman, N. 1975. Fallow deer: Their history, distribution and biology. Terence Dalton. Lavenham, England.

Chew, R.M. and Spencer, E. 1967. Development of metabolic response to cold in young mice of four species. - Comp. Biochem. Physiol. 22: 873-888.

Clara, M. 1937. Zur Histobiologie des Bronchiolarepithels. – Z. Mikrosk. Anat. Forsch. 41: 321-347.

Clements, F., Hope, P., Daniels, C., Chapman, I. and Wittert, G. 1998. Thermogenesis in the marsupial *Sminthopsis crassicaudata*: effect of catecholamines and diet. – Aust. J. Zool. 46: 381-390.

Cockburn, A. and Johnson, C.N. 1988. Patterns of growth. - In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

Collins, L.R. 1973. Monotremes and marsupials. Smithsonian Institution Press. Washington D.C.

Collins, M.H., Kleinerman, J., Moessinger, A.C., Collins, A.H., James, L.S. and Blanc, W.A. 1986. Morphometric analysis of the growth of the normal fetal guinea pig lung. - Anat. Rec. 216: 381-391.

Condon, J.F., Jeyasuria, P., Faust, J.M. and Mendelson, C.R. 2004. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. – Proc. Nat. Acad. Sci. 101 *(14)*: 4978-4983.

Cothran, E.G., Aivaliotis, M.J. and Vandeberg, J.L. 1985. The effects of diet on growth and reproduction in Gray short-tailed opossums (*Monodelphis domestica*). – J. Exp. Zool. 236: 103-114.

Crichton, E.G. 1969. Reproduction in the pseudomyine rodent *Mesembriomys gouldii* (Gray) Muridae. – Australian J. Zool. 17: 785-797.

Crighton, G.W. 1974. The homeothermic status of the neonatal dog. – Nature. 251: 142-144.

Cronin, L. 2000. Australian Mammals. – Envirobook. Annandale, NSW.

Cross, P.C. and Mercer, K.L. 1993. Cell and tissue ultrastructure. A functional perspective. W.H. Freeman and Company. USA.

Crowcroft, P. 1957. The life of the shrew. Reinhardt. London.

Dagg, A.I. and Foster, J.B. 1976. The giraffe. Van Nostrand Reinhold. New York.

Darwin, C. 1859. The Origin of Species. 1st Ed. John Murray. London.

Davis, J.A. 1965. A preliminary report on the reproductive behavior of the small Malayan chevrotain, *Tragulus javanicus*, at the New York Zoo. – Int. Zoo Yearb. 5: 42-44.

Davis, R. 1969. Growth and development of young pallid bats, *Antrozous pallidus*. – J. Mammal. 50: 729-736.

Davies, P., Reid, L., Lister, G. and Pitt, B. 1988. Postnatal growth of the sheep lung: A morphometric study. – Anat. Rec. 220: 281-286.

Dawson, T.J. and Hulbert, A.J. 1970. Standard metabolism, body temperature and surface areas of Australian marsupials. – Am. J. Physiol. 218: 1233-1238.

De, R.N. 1947. A procession of musk shrews. - J. Bombay Nat. Hist. Soc. 47:373.

Dijk van, M.A.M., Madsen, O., Catzeflis, F., Stanhope, M.J., de Jong, W. and Pagel, M. 2001. Protein sequence signatures support the African clade of mammals. – Proc. Natl. Acad. Sci. 98 *(1)*: 188-193.

Dittrich, L. 1969. Birth weights and weight increases of African antelopes born in the Hanover Zoo. – Int. Zoo Yearb. 9: 118-120.

Dittrich, L. 1976. Age of sexual maturity in the hippopotamus. – Int. Zoo. Yearb. 16: 171-173.

Dividich, J.Le and Noblet, J. 1981. Colostrum intake and thermoregulation in the neonatal pig in relation to environmental temperature. – Biol. Neonate. 40: 167-174.

Docimo, S.G., Crone, R.K., Davies, P. Reid, L., Retik, A.B. and Mandell, J. 1991. Pulmonary development in the fetal lamb: A morphometric study of the alveolar phase. – Anat. Rec. 229: 495-498.

Dolbeer, R.A. 1973. Reproduction in the red squirrel (*Tamiasciurus hudsonicus*) in Colorado. – J. Mammal. 54: 536-540.

Dryden, G.L. 1968. Growth and development of *Suncus murinus* in captivity on Guam. – J. Mamm. 49 *(1)*: 51-62.

Dryden, G.L. and Anderson, R.R. 1978. Milk composition and ist relation to growth rate in the musk shrew, *Suncus murinus*. – Comp. Biochem. Physiol. A. 60: 213-216.

Dryden, G.L., Gebczynski, M. and Douglas, E.L. 1974. Oxygen consumption by nursling and adult Musk shrews. – Acta Theriol. 19 *(30)*: 453-461.

Eadie, W.R. 1939. A contribution to the biology of *Parascalops breweri*. – J. Mammal. 20: 150-173.

Edson, J.L. and Hull, D. 1977. The effect of maternal starvation on the metabolic response to vold of the newborn rabbit. – Pediatr. Res. 11: 793-795.

Egoscue, H.J. 1957. The desert woodrat: A laboratory colony. – J. Mammal. 38: 472-481.

Eibl-Eibesfeldt, I. 1951. Gefangenschaftsbeobachtungen an der persichen Wüstenmaus (*Meriones persicus persicus* Blanford). – Z. Tierpsychol. 8: 400-423.

Eisenberg, J.F. 1963. The behavior of heteromyid rodents. – Univ. California Publ. Zool. 69: 1-100.

Eisenberg, J.F. 1967. Comparative studies on the behavior of rodents with special emphasis on the evolution of social behavior, Part I. – Proc. U.S. Nat. Mus. 122 *(3597)*: 1-55.

Eisenberg, J.F. 1975. Tenrecs and solenodons in captivity. – Int. Zoo Yearb. 15: 6-12.

Eisenberg, J.F. 1983. The Mammalian Radiations – An analysis of Trends in Evolution, Adaptation, and Behavior. University of Chicago Press, Chicago.

Eisenberg, J.F. 1988. Reproduction in polyprotodont marsupials and similar-sized eutherians with a speculation concerning the evolution of litter size in mammals. – In: Boyce, M.S. (ed.). Evolution of life history of mammals – Theory and patterns. Yale University Press. New Haven. London.

Eisenberg, J.F. and Maliniak, E. 1973. Breeding and captive maintenance of the lesser bamboo rat, *Cannomys badius*. – Int. Zoo Yearb. 13: 204-207.

Encke, W. 1965. Aufzucht von Borstengürteltieren, *Chaetopharctus villosus*. – Zool. Gart. 31 *(1/2)*: 88-90.

Engel, S. 1962. Lung Structure. Charles C. Thomas. Springfield, Illinois.

Ewer, R.F. 1973. The carnivores. Cornell University Press. Ithaca.

Fadem, B.H., Trupin, G.L., Maliniak, E., VandeBerg, J.L. and Hayssen, V. 1982. Care and Breeding of the Gray, Short-Tailed Opossum (*Monodelphis domestica*). – Lab. Anim. Sci. 32: 405-490.

Fahey, J.T. and Lister, G. 1989. Resonse to low cardiac output: Developmental differences in Metabolism during oxygen deficit and recovery in lambs. – Ped. Res. 26 *(3)*: 180-187.

Farber, J.P., Hultgren, H.N. and Tenney, S.M. 1972. Development of the chemical control of breathing in the virginia opossum. – Respir. Physiol. 14: 267-277.

Findlay, L. 1982. The mammary glands of the tammar wallaby (*Macropus eugenii*) during pregnancy and lactation. – J. Reprod. Fertil. 65: 59-66.

Findlay, L. and Renfree, M.B. 1984. Growth, development and secretion of the mammary gland of macropodid marsupials. – Symp. Zool. Soc. Lond. 51: 403-432.

Fitzgerald, L.R. 1953. The oxygen consumption of neonatal mice. – J. Exp. Zool. 124 *(3)*: 415-425.

Fleay, D. 1944. We breed the platypus. Robertson and Mullens. Melbourne.

Flux, J.E.C. 1967. Reproduction and body weights of the hare, *Lepus europaeus* Pallas, in New Zealand. – New Zealand J. Sci. 10: 357-401.

Flux, J.E.C. 1970. Life history of the mountain hare (*Lepus timidus scoticus*) in northeastern Scotland. – J. Zool. 161: 45-123.

Fons, R. 1973. Modalites de la reproduction et developpement postnatal en captivite chez *Suncus etruscus* (Savi 1822). – Mammalia 37 *(2)*: 288-325.

Foresman, K.R. 1994. Comparative embryonic development of the Soricidae. – In: Merritt, F., Kirkland, G.L. and Rose, R.K. (eds.). Advances in the biology of shrews. Carnegie Museum of Natural History. Spec. Publ. 18. Pittsburgh.

Frädrich, H. 1966. Einige Verhaltensbeobachtungen am Moschustier (*Moschus moschiferus* L.). – Zool. Gart. 33 *(1/3)*: 65-78.

Frappell, P.B. and Mortola, J.P. 2000. Respiratory function in a newborn marsupial with skin gas exchange. – Resp. Biol. 120: 35-45.

Frappell, P., Saiki, C. and Mortola, J.P. 1991. Metabolism during normoxia, hypoxia and recovery in the newborn kitten. – Resp. Physiol. 86: 115-124.

Frappell, P., Lanthier, C., Baudinette, R.V. and Mortola, J.P. 1992. Metabolism and ventilation in acute hypoxia: a comparative analysis in small mammalian species. – Am. J. Physiol. 262: R1040-R1046.

French, N.R., Stoddard, D.M. and Bobek, B. 1975. Patterns of demography in small mammal populations. – In: Golley, K. Petrusewicz, K. and Ryszkowski, L. (eds.). Small mammals: Their productivity and population dynamics. Cambridge University Press. London.

Fuchs, E. 1999. Tree Shrews. – In: Poole, T. and English, P. (eds.). The UFAW Handbook on the care and management of laboratory animals. Blackwell Science, Oxford.

Gaillard, J.-M., Pontier, D., Allaine, D., Loison, A., Herve, J.-C. and Heizmann, A. 1997. Variation in growth form and precocity at birth in eutherian mammals. – Proc. R. Soc. Lond. B. 264: 859-868.

Gates, D.R. 1974. The ontogeny of thermoregulation in the cottontail rabbit (*Sylvilagus floridanus*). M.S. thesis, Western Illinois University.

Gauthier-Pilters, H. 1974. The behaviour and ecology of camels in the Sahara, with special reference to normadism and water management. – In: Geist, V. and Walther, F. (eds.). The behaviour of ungulates and ist relation to management. IUCN Publications. No. 24. Morges.

Geiser, F., Matwiejczyk, L. and Baudinette, R.V. 1986. From Ectothermy to Heterothermy: the energetics of the Kowari, *Dasyuroides byrnei* (Marsupialia: Dasyuridae). – Physiol. Zool. 59 *(2)*: 220-229.

Geiser, F. 1988. Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? – J. Comp. Physiol. B. 158: 25-37.

Gelineo, S. 1962. Razvitak hemijske termoregulacije u poljske voluharice *Microtus arvalis*. – Pall. Rad Jugosl. Akad. Znan. Umjet. 329: 41-64.

Gemmell, R.T. 1986. Lung development in the marsupial bandicoot, *Isoodon macrourus*. – J. Anat. 148: 193-204.

Gemmell, R.T. and Cepon, G. 1993. The development of thermoregulation in the marsupial brushtail possum *Trichosurus vulpecula*. – Comp. Biochem. Physiol. A. 106 *(2)*: 167-173.

Gemmell, R.T. and Little, G.J. 1982. The structure of the lung of the newborn marsupial bandicoot, *Isoodon macrourus*. – Cell Tissue Res. 223 *(2)*: 445-453.

Gemmell, R.T. and Nelson, J. 1988. The ultrastructure of the lung of two newborn marsupial species, the northern native cat, *Dasyurus hallucatus*, and the brushtail possum, *Trichosurus vulpecula*. – Cell Tissue Res. 252: 683-685.

Gemmell, R.T. and Selwood, L. 1994. Structural development in the newborn marsupial, the stripe-faced dunnart, *Sminthopsis macroura*.- Acta Anat. 149: 1-12.

Gemmell, R.T., Bell, A.W. and Alexander, G. 1972. Morphology of adipose cells in lambs at birth and during subsequent transition of brown to white adipose tissue in cold and warm conditions. – Am. J. Anat. 133: 143-163.

Gemmell, R.T., Cepon, G. and Barnes, A. 1987. The development of thermoregulation in the rat kangaroo, *Potorous tridactylus*. – Comp. Biochem. Physiol. A. 88 *(2)*: 257-261.

Gemmell, R.T., Veitch, C. and Nelson, J. 2002. Birth in marsupials. – Comp. Biochem. Physiol. B. 131: 621-630.

Gerrish, C.J., Onischak, C.M. and Alberts, J.R. 1998. Acute, early thermal experience alters weaning onset in rats. – Physiol. Behav. 64 *(4)*: 463-474.

Godfrey, G. and Crowcroft, P. 1960. The life of the mole. Museum Press. London.

Goldner, J. 1938. A modification of the masson trichromtechnique for routine laboratory purpose. – Am. J. Pathol. 14: 237-243.

Gopalakrishna, A. 1969. Gestation period in some Indian bats. – J. Bombay Nat. Hist. Soc. 66: 317-322.

Grant, T.R. 1995. The platypus, a unique mammal. 3rd ed. UNSW Press. Sydney.

Green, B. and Merchant, J. 1988. The composition of marsupial milk. - In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

Green, B., Griffiths, M. and Leckie, R.M.C. 1983. Qualitative and quantitative changes in milk fat during lactation in the tammar wallaby (*Macropus eugenii*). – Aust. J. Biol. Sci. 36: 455-461.

Green, B., Merchant, J.C. and Newgrain, K. 1988. Milk composition and energetics of growth in pouch young of the tammar wallaby *Macropus eugenii*. – Aust. J. Zool. 36: 217-227.

Griffiths, M. 1968. Echidnas. Pergamon Press. New York.

Griffiths, M. 1978. The Biology of the Monotremes. Academic Press. New York, San Francisco, London.

Grünwald, A. and Möhres, F.P. 1974. Beobachtungen zur Jugendentwicklung und Karawanenbildung bei Weißzahnspitzmäusen (Soricidae-Crocidurinae). – Z. Säugetierkunde 39: 321-337.

Gucwinska, H. 1971. Development of six-banded armadillos *Euphractus sexcinctus* at Wroclaw Zoo. – Int. Zoo Yearb. 11: 88-89.

Gucwinska, H. and Gucwinska, A. 1968. Breeding the *Zanzibar galago*, *Galago senegalensis zanzibaricus* in the Wroclaw Zoo. – Int. Zoo Yearb. 8: 111-114.

Gulisano, M., Pacini, P., Marceddu, S. and Orlandini, G.E. 1995. Scanning electron microscopic evaluation of the alterations induced by polluted air in the rabbit bronchial epithelium. – Ann. Anat. 177: 125-131.

Gusztak, R.W. and Campbell, K.L. 2004. Growth, development and maintenance of American water shrews (*Sorex palustris*) in captivity. – Mammal Study. 29: 65-72.

Hackländer, K., Arnold, W. and Ruf, T. 2002. Postnatal development and thermoregulation in the precocial European hare (*Lepus europaeus*). - J. Comp. Physiol. B. 172: 183-190.

Hall, A. III, Persing, R.L., White, D.C. and Rickets, R.T. 1967. Mystromys albicaudatus (the African white tailed rat) as a laboratory species. – Lab. Anim. Care. 17: 180-188.

Hall, E.R. and Kelson, K.R. 1959. The mammals of North America. 2 vols. Ronald Press. New York.

Hall, L.S. 1987. The embryology of the marsupial, *Isoodon macrourus*, with particular reference to fetation. PhD thesis. Univ. Queensland.

Hamilton, J.E. 1934. The southeastern sealion *Otaria byronia* (de Blainville). – Discov. Rep. 8: 269-318.

Hamilton, W.J. 1944. The biology of the little short tailed shrew *Cryptotis parva*. – J. Mammal. 25: 1-7.

Harder, J.D., Hsu, M.J. and Garton, D.W. 1996. Metabolic rates and body temperature of the Gray short-tailed opossum (*Monodelphis domestica*) during gestation and lactation. – Physiol. Zool. 69 *(2)*: 317-339.

Harris, H.F. 1898. A new method of ripening haematoxylin. – Micr. Bull. 1898: 47.

Hart, J.S., Heroux, O., Cottle, W.H. and Mills, C.A. 1961. The influence of climate on metabolic and thermal responses of infant caribou. – Can. J. Zool. 39: 845-856.

Hartman, D.S. 1971. Behavior and ecology of the Florida manatee, *Trichechus monatus latirostris* (Harlan) at Crystal River, Citrus County. PhD. Diss. Cornell University.

Hayssen, V. and Lacy, R.C. 1985. Basal metabolic rates in mammals: Taxonomic differences in the allometry of SMR and body mass. – Comp. Biochem. Physiol. A 81*(4)*: 741-754.

Hedges, S.B. 2001. Afrotheria: Plate tectonics meets genomics. - Proc. Natl. Acad. Sci. 98:1-2.

Heidenhain, M. 1915. Über die Mallorysche Bindegewebsfärbung mit Karmin und Azokarmin als Vorfarben. – Z. w. M. 32: 361-372.

Heldmair, G. and Ruf, T. 1992. Body temperature and metabolic rate during natural hypothermia in endotherms. - J. Comp. Physiol. B. 162: 696-706.

Hellwing, S. 1973. The postnatal development of the white-toothed shrew *Crocidura russula monacha* in captivity. – Z. Säugetierk. 38: 257-270.

Helm, J.D. 1973. Reproductive biology of *Tylomys* and *Ototylomys*. Ph.D. diss. Michigan State University.

Hemmer, H. 1976. Gestation period and postnatal development in felids. – In: Eaton, R.L. (ed.). The world's cats. Vol. 3. No. 2. Contributions to biology, ecology, behavior and evolution. Carnivore Research Institute, Burke Museum, University of Washington. Seattle.

Hennig, W. 1950. Grundzüge einer Theorie der Phylogenetischen Systematik. Deutscher Zentralverlag. Berlin.

Hertenstein, B., Zimmermann, E. und Rahmann, H. 1987. Zur Reproduktion und ontogenetischen Entwicklung von Spitzhörnchen (*Tupaia belangeri*). – Z. Köln. Zoo 30 *(4)*: 119-133.

Herter, K. 1957. Das Verhalten der Insektivoren. – Handb. Zool. Bd. 8 (9). 19 *(10)*: 1-50.

Heuser, C.H. 1921. The early establishment of intestinal nutrition in the opossum-the digestive tract just before and soon after birth. – Am. J. Anat. 23: 341-356.

Hick, U. 1969. Successful raising of a pudu *Pudu pudu* at Cologne Zoo. – Int. Zoo Yearb. 9: 110-112.

Hill, R.W. 1970. The ontogeny of homeothermy in neonatal *Peromyscus leucopus noveboracensis*. PhD. Dissertation. University of Michigan.

Hill, R.W. 1992. The Altricial/Precocial contrast in the thermal relations and energetics of small mammals. – In: Tomasi, T.E. and Horton, T.H. (eds.). Mammalian energetics. Interdisciplinary views of metabolism and reproduction. Comstock Publishing Associates. Ithaca. London.

Hill, J.P. 1910. Contributions to the embryology of the Marsupialia. 4. The early development of the Marsupialia with special reference to the native cat (*Dasyurus viverrinus*). – Quart. J. Microscop. Sci. 56: 1-134.

Hill, J.P. and Hill, W.C.O. 1955. The growth stages of the pouch young of the native cat (*Dasyurus viverrinus*) together with observations on the anatomy of the newborn young. – Trans. Zool. Soc. Lond. 28: 349-452.

Hislop, A., Howard, S. and Fairweather, V.I. 1984. Morphometric studies on the structural development of the lung in *Macaca fascicularis* during fetal and postnatal life. – J. Anat. 138 *(1)*: 95-112.

Hissa, R. 1968. Postnatal development of thermoregulation in the Norwegian lemming and the golden hamster. – Ann. Zool. Fenn. 5: 345-383.

Hitchcock, K.R. 1980. Lung development and the pulmonary surfactant system: Hormonal influences. – Anat. Rec. 198: 13-34.

Hopson, J.A. 1973. Endothermy, small size and the origin of mammalian reproduction. – Amer. Naturalist. 107: 446-452.

Horowitz, I. and Sanchez-Villagra, M.R. 2003. A morphological analysis of marsupial mammal higher-level phylogenetic relationships. – Cladistics. 19: 181-212.

Horwich, R.H. 1972. The ontogeny of social behavior in the gray squirrel (*Sciurus carolinensis*). – Z. Tierpsychol. Suppl. 8: 1-103.

Hsu, M.J., Garton, D.W. and Harder, J.D. 1999. Energetics of offspring production: a comparison of a marsupial (*Monodelphis domestica*) and a eutherian (*Mesocricetus auratus*). – J. Comp. Physiol. B 169: 67-76.

Hudson, J.W. 1974. The estrous cycle, reproduction, growth and development of temperature regulation in the pygmy mouse, *Baiomys taylori*. – J. Mammal. 55: 572-588.

Hughes, R.L. and Hall, L.S. 1988. Structural adaptations of the newborn marsupial. – In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

Hulbert, A.J. 1988. Metabolism and the development of endothermy. - In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

Hull, D. 1965. Oxygen consumption and body temperature of newborn rabbits and kittens exposed to cold. – J. Physiol. 177: 192-202.

Hull, D. and Segall, M.M. 1965. Heat production in the newborn rabbit and fat content of the brown adipose tissue. – J. Physiol. 181: 468-477.

Hunt, T.P. 1959. Breeding habits of the wamp rabbit with notes on its life history. – J. Mammal. 40: 82-91.

Hutterer, R. 1976. Beobachtungen zur Geburt und Jugendentwicklung der Zwergspitzmaus, *Sorex minutus* L. (Soricidae: Insectivora). – Z. Säugetierk. 41 *(1)*: 1-22.

Ingles, L.G. 1965. Mammals of the Pacific states. Stanford University Press. Stanford.

Innes, D.G.L. 1994. Life histories of the Soricidae: a review. – In: Merritt, F., Kirkland, G.L., Rose, R.K. (eds.). Advances in the biology of shrews. Carnegie Museum of Natural History. Vol. 18. Pittsburgh.

Iovannitti, B., Pirie, H.M. and Wright, N.G. 1985. Scanning electron microscopic study of the lower respiratory tract in calves and adult cattle. – Res. Vet. Sci. 38: 80-87.

Ito, T. and Kanisawa, M. 1990. Endocrine cells and brush cells at the bronchio-alveolar junctions of neonatal syrian hamster lungs. – J. Morph. 206: 217-223.

IUPS Thermal Commission. 2003. Glossary of terms for thermal physiology. – J. Therm. Biol. 28: 75-106.

Iversen, J.A. and Krog, J. 1973. Heat production and body surface area in seals and sea otters. – Norw. J. Zool. 21: 51-54.

Jacobsen, N.H.G. 1982. A note of mating and neonate development of the South african hedgehog (*Erinaceus frontalis*). – Säugetierk. Mitt. 30 *(3)*: 199-200.

Janke, O., Tzschentke, B., Höchel, J. and Nichelmann, M. 2002. Metabolic responses of chicken and muscovy duck embryos to high incubation temperatures. – Comp. Biochem. Physiol. A 131: 741-750.

Janssens, P.A., Hulbert, A.J. and Baudinette, R.V. 1997. Development of the pouch young from birth to pouch vacation. – In: Saunders, N.R. and Hinds, L.A. (eds.). Marsupial Biology-Recent Research, New Perspectives. University of New South Wales Press Ltd.

Ji, Q., Luo, Z.-X., Yuan, C.-X; Wible, J.R., Zhang, J.-P. and Georgi, J.A. 2002. The earliest known eutherian mammal. – Nature. 416: 816-822.

Johnson, G.E. 1931. Early life of the thirteen-lined ground squirrel. – Trans. Kansas Acad. Sci. 34: 282-290.

Johnson, K.R. and Hickey, L.J. 1990. Megafloral change across the Cretaceous/Tertiary boundary in the northern Great Plains and Rocky mountains, USA. – Geol. Soc. Am. Spec. Pap. 247: 433-444.

Johnston, R.F. and Rudd, R.L. 1957. Breeding the salt marsh shrew. – J. Mammal. 38: 157.

Johnston, S.D. and Daniels, C.B. 2001. Development of the pulmonary surfactant system in non-mammalian amniotes. – Comp. Biochem. Physiol. A. 129: 49-63.

Kahwa, C.K.B., Balemba, O.B. and Assey, R.J. 2000. The pattern of ciliation and the development of the epithelial lining of the respiratory tract in the neonatal kid: a scanning electron microscopic study. – Small Rum. Res. 37: 27-34.

Keller, G., Adatte, T., Stinnesbeck, W., Rebolledo-Vieyra, M., Fucugauchi, J.U., Kramar, U. and Stüben, D. 2004. Chicxulub impact predates the K-T boundary mass extinction. - Proc. Natl. Acad. Sci. 101 *(11)*: 3753-3758.

Kemp, T.S. 2005. The Origin and Evolution of Mammals. Oxford University Press. Oxford.

Kielan-Jaworowska, Z. and Dashzeveg, D. 1989. Eutherian mammals from the Early Cretaceous of Mongolia. – Zool. Script. 18: 347-355.

Kikkawa, Y., Kaibara, M., Motoyama, E.K., Orzatesi, M.M. and Cook, C.D. 1971. Morphologic development of fetal rabbit lung and its acceleration with cortisol. – Amer. J. Path. 64: 423-442.

Kirkpatrick,T.H. 1965. Studies of Macropodidea in Queensland. 2. Age estimation in the grey kangaroo, the red kangaroo, the eastern walleroo and the red-necked wallaby, with notes on dental abnormalities. – Queensl. J. Agricult. Anim. Sci. 22: 301-317.

Kleiber, M. 1961. The Fire of life. Wiley. New York.

Kleiman, D.G. 1969. Maternal care, growth rate, and development in the noctule, pipistrelle and serotine bats. – J. Zool. (London) 157 *(2)*: 187-212.

Kleiman, D.G. 1972. Maternal behaviour of the green acouchi (*Myoprocta pratti* Pocock), a South American caviomorph rodent. – Behaviour 43 *(1-4)*: 48-84.

Kleiman, D.G. and Davis, T.M. 1978. Ontogeny and maternal care. – In: Baker, R.J., Jones, J.K. and Carter, D.C. (eds.). Biology of the New World Family Phyllostomatidae. Part III. Spec. Publ. Mus. Texas Tech Press. Lubbock. Texas.

Knipe, T. 1957. The javelina in Arizona. State of Arizona Game and Fish Department. Phoenix.

Koffler, B.R. 1972. *Meriones crassus*. – Mammal. Spec. 9: 1-4.

Koford, C.B. 1957. The vicuna and the puna. – Ecol. Monogr. 27: 153-219.

Königsmann, E. 1975. Termini der phylogenetischen Systematik. – Biol. Rundschau. 13: 99-115.

Koteja, P. 1987. On the relation between basal and maximum metabolic rate in mammals. – Comp. Biochem. Physiol. A. 87 *(1)*: 205-208.

Kozlowski, J. and Konarzewski, M. 2004. Is West, Brown and Enquist's model of allometric scaling mathematically correct and biologically relevant? – Funct. Ecol. 18: 283-289.

Kraus, D.B. and Fadem, B.H. 1987. Reproduction, Development and Physiology of the Gray Short-tailed Opossum (*Monodelphis domestica*). – Lab. Anim. Sci. 37*(4)*: 478-483.

Krause, W.J. and Leeson, C.R. 1973. The postnatal development of the respiratory system of the opossum. I. Light and scanning electron microscopy. – Am. J. Anat. 137: 337-356.

Krause, W.J. and Leeson, C.R. 1975. Postnatal development of the respiratory system of the opossum. II. Electron microscopy of the epithelium and pleura. – Acta Anat. 92: 28-44.

Krause, W.J., Cutts, J.H. and Leeson, C.R. 1979. Morphological observations on the mesonephros in the postnatal opossum, *Didelphis virginiana*. – J. Anat. 129 *(2)*: 377-397.

Kuhn, H.-J. and Schwaier, A. 1973. Implantation, early placentation, and chronology of embryogenesis in *Tupaia belangeri*. – Z. Anat. Entwickl.-Gesch. 142: 315-340.

Künkele, J. and Trillmich, F. 1997. Are precocial young cheaper? Lactation energetics in the Guinea pig. – Physiol. Zool. 70 *(5)*: 589-596.

Kurta, A. and Kunz, T.H. 1987. Size of bats at birth and maternal investment during pregnancy. - Symp. Zool. Soc. Lond. 57: 79-106.

Langston, C., Kida, K., Reed, M. and Thurlbeck, W.M. 1984. Human lung growth in late gestation and in the neonate. – Am. Rev. Respir. Dis. 129: 607-613.

Larkin, P. and Roberts, M. 1979. Reproduction in the ring-tailed mongoose *Galidea elegans* at the National Zoological Park, Washington. – Int. Zoo Yearb. 19: 188-192.

Layne, J.N. 1968. Ontogeny of *Peromyscus*. – In. King, J.A. (ed.). Biology of *Peromyscus* (Rodentia). Special Publication American Society Mammalogists.

Lechleitner, R.R. 1959. Sex ratio, age classes and reproduction of the black-tailed jack rabbit. – J. Mammal. 40: 63-81.

Lechner, A.J. and Banchero, N. 1982. Advanced pulmonary development in newborn Guinea pigs (*Cavia porcellus*). – Am. J. Anat. 163: 235-246.

Lee, A.K. and Cockburn, A. 1985. The evolutionary ecology of marsupials. Cambridge University Press. Cambridge.

Lent, P.C. 1966. Calving and related social behavior in the barren-ground caribou population. – Anim. Behav. 13: 259-264.

Leraas, H.J. 1938. Observations on the growth and behavior of harvest mice. – J. Mammal. 19: 441-444.

Leutenegger, W. 1973. Maternal fetal weight relationships in primates. – Folia Primat. 20: 280-293.

Lillegraven, J.A. 1975. Biological considerations of the marsupial-placental dichotomy. – Evolution. 29: 707-722.

Lillegraven, J.A. 1979. Reproduction in Mesozoic mammals. – In: Mesozoic Mammals: Lillegraven, J.A., Kielan- Jaworowska, Z. and Clemens, W.A. (eds.). The First Two-thirds of Mammalian History. University of California Press. Berkeley.

Linsdale, J.M. and Tevis, L.P. 1951. The dusky-footed wood rat. University of California Press. Berkeley.

Linsdale, J.M. and Tomich, P.Q. 1953. A herd of mule deer. University of California Press. Berkeley.

Linzey, D.W. and Linzey, A.V. 1967. Growth and development of the golden mouse, *Ochrotomys nuttalli nuttalli*. – J. Mammal. 48: 445-458.

Lochbrunner, A. 1956. Beiträge zur Biologie des Syrischen Goldhamsters *Mesocricetus auratus* (NEHRING). – Zool. Jahrb. 66 *(2/3)*: 389-428.

Loudon, A., Rothwell, N. and Stock, M. 1985. Brown fat, thermogenesis and physiological birth in a marsupial. – Comp. Biochem. Physiol. A. 81 *(4)*: 815-819.

Luckett, W.P. 1980. Comparative biology and evolutionary relationships of tree shrews. Plenum Press, New York.

Luckett, W.P. 1994. Suprafamilal relationships within Marsupialia: resolution and discordance from multidisciplinary data. – J. Mamm. Evol. 2: 225-283.

Ludwig, E. 1957. Embryologische Beobachtungen an den Harnorganen der Maus und des Goldhamsters. – Acta Anat. 29: 1-19.

Luo, Z.-X., Ji, Q., Wible, J.R. and Yuan, C.-X. 2003. An early Cretaceous tribosphenic mammal and metatherian evolution. – Science. 302: 1934-1940.

Lynch, C.B. 1974. Environmental modification of nest-building in the white-footed mouse, Peromyscus *leucopus*. – Anim. Behav. 22: 405-409.

MacArthur, R.A. and Humphries, M.M. 1999. Postnatal development of thermoregulation in the semiaquatic muskrat (*Ondatra zibethicus*). – Can. J. Zool. 77: 1521-1529.

MacDonald, D. 2002. The new encyclopedia of mammals. Oxford University Press, Oxford.

MacFarlane, P.M. and Frappell, P.B. 2001. Convection requirement is established by total metabolic rate in the newborn tammar wallaby. – Resp. Physiol. 126: 221-231.

Maier, W. 1999. On the evolutionary biology of early mammals – With methodological remarks on the interaction between ontogenetic adaptation and phylogenetic transformation. – Zool. Anz. 238: 55-74.

Makanya, A.N., Haenni, B. and Burri, P.H. 2003. Morphometry and allometry of the postnatal lung development in the quokka wallaby (*Setonix brachyurus*): a light microscopic study. – Resp. Physiol. 134: 43-55.

Maliniak, E. and Eisenberg, J.F. 1971. The breeding of *Proechimys semispinosus* in captivity. – Int. Zoo Yearb. 11: 93-98.

Mallinson, J. 1973. The reproduction of the African civet *Viverra civetta* at Jersey Zoo. – Int. Zoo Yearb. 13: 147-150.

Maloney, J.E. 1984. The development of the respiratory system in placental mammals. – Persp. Vert. Sci. 3: 373-387.

Marshall, L.G., Case, J.A. and Woodburne, M.O. 1990. Phylogenetic relationships of the families of marsupials. – In: Genoways, H.H. (ed.). Current Mammalogy. Vol.2. Plenum Press, New York.

Martin, R.A. 1980. Body mass and basal metabolism of extinct mammals. – Comp. Biochem. Physiol. A. 66: 307-314.

Martin, R.D. 1967. Reproduction and ontogeny in tree shrews (*Tupaia belangeri*), and reference to their general behavior and taxonomic relationships. – Z. f. Tierpsychol. 25 *(4)*: 409-532.

Martin, R.J. 1973. Growth curves for bushy-tailed woodrats based upon animals raised in the wild. – J. Mammal. 54: 517-518.

Mate, K.E., Gentle, R.L., Fletcher, T.P. and Rodger, J.C. 1999. Australian Marsupials. – In: Poole, T. and English, P. (eds.). The UFAW Handbook on the care and management of laboratory animals. Blackwell Science, Oxford.

McCrady, J.E. 1938. The embryology of the opossum. – Am. Anat. Mem. 16: 1-226.

McKay, G.M. 1973. The ecology and behavior of the Asiatic elephant in southeastern Ceylon. – Smithsonian Contrib. Zool. 125: 1-113.

McNab, B.K. 1980. Food habits, energetics, and the poulation biology of mammals. – Am. Nat. 116: 106-124.

McNab, B.K. 1986. Food habits, energetics, and the reproduction of marsupials. – J. Zool. Lond. A 208: 595-614.

Medway, Lord. 1972. Reproductive cycles of the flat-headed bats, *Tylonycteris pachypus* and *T. robustula* (Chiroptera: Vespertilioninae) in a humid equatorial environment. – Zool. J. Linnean Soc. 51: 33-61.

Melton, D.A. 1976. The biology of aardvarks (Tubulidentata-Orycteropodidae). – Mammal. Rev. 6 *(2)*: 75-88.

Mendelssohn, H. 1965. Breeding the Syrian hyrax, *Procavia capensis syriaca* Schreber 1784. – Int. Zoo Yearb. 5: 116-125.

Mentis, M.T. 1972. A review of some life history features of the large herbivores of Africa. – Lammergeyer 16: 1-89.

Merchant, J.C. and Sharman, G.B. 1966. Observations on the attachment of marsupial pouch young to the teats and on the rearing of pouch young by foster-mothers of the same or different species. – Austr. J. Zool. 14: 593-609.

Mercurio, A.R. and Rhodin, J.A.G. 1984. An electron microscopic study on the type I pneumocyte in the cat: Postnatal morphogenesis. – J. Morph. 182: 169-178.

Meritt, D.A. 1976. The La Plata three-banded armadillo (*Tolypeutes matacus*) in captivity. – Int. Zoo Yearb. 16: 153-156.

Messer, M., Weiss, A.S., Shaw, D.C. and Westerman, M. 1998. Evolution of the monotremes: phylogenetic relationship to marsupials and eutherians, and estimation of divergence dates based on α-lactalbumin amino acid sequences. – J. Mamm. Evol. 5: 95-105.

Meyer, B.J. and Meyer, R.K. 1944. Growth and reproduction of the cotton rat, *Sigmodon hispidus hispidus*, under laboratory conditions. – J. Mammal. 25: 107-129.

Miller, K., Rosenmann, M. and Morrison, P. 1976. Oxygen uptake and temperature regulation of young harbour seals (*Phoca vitulina* Richardi) in water. – Comp. Biochem. Physiol. A. 54: 105-107.

Mills, G. and Hes, L. 1999. Säugetiere des südlichen Afrikas. Eine illustrierte Enzyklopädie. Könemann. Köln.

Mohr, E. 1954. Die freilebenden Nagetiere Deutschlands und der Nachbarländer. Gustav Fischer. Jena.

Mohr, E. 1970. Wilde Schweine. Ziemsen. Wittenberg.

Montgomery, G.G. and Sunquist, M.E. 1974. Contact-distress calls of young sloths. – J. Mammal. 55: 211-213.

Moore, K.L. 1977. The developing human. 2nd ed. Saunders. Philadelphia.

Morrison. P.R., Ryser, F.A. and Strecker, R.L. 1954. Growth and development of temperature regulation in the tundra redback vole (*Clethrinomys rutilus dawsoni*). – J. Mamm. 35: 376-386.

Mortola, J.P. 1991. Hamsters versus rats: ventilatory responses in adults and newborns. – Resp. Physiol. 85: 305-317.

Mortola, J.P. 2001. Respiratory physiology of newborn mammals - a comparative perspective. John Hopkins University Press. Baltimore.

Mortola, J.P. and Lanthier, C. 1996. The ventilatory and metabolic response to hypercapnia in newborn mammalian species. – Resp. Physiol. 103: 263-270.

Mortola, J.P., Frappell, P.B., Dotta, A., Matsuoka, T., Fox, G., Weeks, S. and Mayer, D. 1992. Ventolatory and metabolic responses to acute hyperoxia in newborns. – Am. Rev. Respir. Dis. 146: 11-15.

Mortola, J.P., Frappell, P.B. and Woolley, P.A. 1999. Breathing through skin in a newborn mammal. – Nature. 397: 660.

Muizon, C.de, Cifelli, R.L. and Cespedes Paz, R. 1997. The origin of the dog-like borhyaenoid marsupials of South America. – Nature. 389: 486-489.

Müller, F. 1969. Zur frühen Evolution der Säugerontogenesetypen: Versuch einer Rekonstruktion aufgrund der Ontogeneseverhältnisse bei den Marsupialia. – Acta Anat. 74: 297-404.

Müller, F. 1972. Zur stammesgeschichtlichen Veränderung der Eutheria-Ontogenesen. – Re. Suisse Zool. 79 *(1)*: 1-97.

Müller-Schwarze, D. and Müller-Schwarze, C. 1973. Behavioral development of hand-reared pronghorn *Antilocapra americana*. – Int. Zoo Yearb. 13: 217.

Murphy, W.J., Eizirik, E., Johnson, W.E., Zhang, Y.P., Ryder, O.A. and O'Brien, S.J. 2001. Molecular phylogenetics and the origin of placental mammals. – Nature. 409: 614-618.

Muul, I. 1969. Mating behavior, gestation period, and development of *Glaucomys sabrinus*. – J. Mammal. 50 *(1)*: 121.

Nagl, W. 1981. Elektronenmikroskopische Laborpraxis. Springer Verlag, Berlin, Heidelberg, New York.

Nagel, A. 1989. Paradoxial social effects on response to cold during ontogeny of the common white-toothed shrew, *Crocidura russula*. – J. Comp. Physiol. B. 159: 301-304.

Nakakuki, S. 1980. Comparative anatomical studies on the mammalian lung. – Bulletin of the Faculty of Agriculture. 21: 1-73. University of Agriculture and Technology. Tokyo.

Neal, E.G. 1958. The badger. Penguin Books. Harmondsworth.

Nessov, L. and Kielan-Jaworowska, Z. 1991. Evolution of the Cretaceous Asian therian mammals. – Fifth Symposium on Mesozoic Terrestrial Ecosystems and Biota. Extended abstracts. Contributions from the Paleontological Museum. Univ. Oslo 364: 51-52.

Netter, F.H. 1997. Atlas der Anatomie des Menschen. Thieme Verlag. Stuttgart, New York.

Newson, J. 1964. Reproduction and prenatal mortality of snowshoe hares on Manitoulin Island, Ontario. – Canad. J. Zool. 10: 225-267.

Nichelmann, M. and Tzschentke, B. 2002. Ontogeny of thermoregulation in precocial birds. – Comp. Biochem. Physiol. A. 131: 751-763.

Nichelmann, M., Burmeister, A., Janke, O., Höchel, J. and Tzschentke, B. 1998. Avian embryonic thermoregulation: a role of Q10 in interpretation of endothermic reactions. – J. Therm. Biol. 23 *(6)*: 369-376.

Nichelmann, M., Höchel, J. and Tzschentke, B. 1999. Biological rhythms in birds – development, insights and perspectives. – Comp. Biochem. Physiol. A. 124: 429-437.

Nichelmann, M., Janke, O. and Tzschentke, B. 2001. Efficiency of thermoregulation in precocial avian species during the prenatal period. – J. Therm. Biol. 26: 273-280.

Niethammer, G. 1950. Zur Jungenpflege und Orientierung der Hausspitzmaus (*Crocidura russula* Herm.). – Bonn. Zool. Beitr. 1 *(2-4)*: 117-125.

Noblet, J. and Le Dividich, J. 1981. Energy metabolism of the newborn pig during the first 24 h of life. – Biol. Neonate 40: 175-182.

North, D. 1999. The Guinea-Pig. – In: Poole, T. and English, P. (eds.). The UFAW Handbook on the care and management of laboratory animals. Blackwell Science, Oxford.

Novacek, M.J. 1993. Reflections on higher mammalian phylogenetics. – J. Mamm. Evol. 1 *(1)*: 3-30.

Novacek, M.J. and Wyss, A.R. 1986. Higher-level relationships of the recent eutherian orders: Morphological evidence. – Cladistics. 2 *(3)*: 257-287.

Novacek, M.J., Wyss, A.R. and McKenna, M.C. 1988. The major groups of eutherian mammals. – In: Benton, M.J. (ed.). The Phylogeny and Classification of the Tetrapods, Vol.2: Mammals. Systematic Association Special Volume No. 35B. Clarendon Press. Oxford, UK.

Oftedal, O.T. and Iverson, S.J. 1995. Comparative anlaysis of nonhuman milks. A phylogenetic Variation in the gross composition of milks. – In: Jensen, R.G. (ed.). Handbook of milk composition. Academic Press. San Diego. New York. Boston. London. Sydney. Tokyo. Toronto.

Orgeig, S., Daniels, C.B., Johnston, S.D. and Sullivan, L.C. 2003. The pattern of surfactant cholesterol during vertebrate evolution and development: does ontogeny recapitulate phylogeny? – Repr. Fert. Develop. 15: 55-73.

O'Hare, K.H., Reiss, O.K. and Vatter, A.E. 1971. Esterases in developing and adult rat lung. I. Biochemistrical and electron microscopic observations. – J. Histochem. Cytochem. 19: 97-115.

O'Hare, K.H. and Sheridan, M.N. 1970. Electron microscopic observations on the morphogenesis of the albino rat lung, with special reference to pulmonary epithelial cells. – Am. J. Anat. 127: 181-205.

Orgeig, S. and Daniels, C.B. 2001. The roles of cholesterol in pulmonary surfactant: insights from comparative and evolutionary studies. – Comp. Biochem. Physiol. A. 129: 75-89.

Ousey, J.C., McArthur, A.J. and Rossdale, P.D. 1991. Metabolic changes in thoroughbred pony foals during the first 24 h post partum. – J. Reprod. Fert. Suppl. 44: 561-570.

Paris, D.B.B.P., Taggart, D.A., Shaw, G., Temple-Smith, P.D. and Renfree, M.B. 2005. Birth of pouch young after artificial insemination in the tammar wallaby (*Macropus eugenii*). – Biol. Reprod. 72: 451-459.

Pearson, O.P., Koford, M.R. and Pearson, A.K. 1952. The reproduction of the lump-nosed bat (*Corynorhinus rafinesquei*) in California. – J. Mammal. 33: 273-320.

Penny, D. and Hasegawa, M. 1997. The platypus put in its place. – Nature. 387: 549-550.

Petter, F. 1961. Repartition geographique et ecologie des rongeurs desertique. – Mammalia 24: 1-219.

Plattner, H. und Hentschel, J. 1997. Taschenlehrbuch Zellbiologie. – Georg Thieme Verlag, Stuttgart.

Plopper, C.G., Alley, J.L., Serabjit-Singh, C. and Philpot, R.M. 1983. Cytodifferentiation of the nonciliated bronchiolar epithelial (Clara) cell during rabbit lung maturation: An ultrastructural and morphometric study. – Am. J. Anat. 167: 329-357.

Poczopko, P. 1971. Metabolic levels in adult homeotherms. – Acta Theriol. 16 *(1)*: 1-21.

Poglayen-Neuwall, I. 1962. Beiträge zu einem Ethogramm des Wickelbären (*Potos flavus* Schreber). – Z. Säugetierk. 27 *(1)*: 1-44.

Poole, W.E., Simms, N.G., Wood, J.T. and Lubulwa, M. 1991. Tables for age determination of the kangaroo island wallaby (tammar), *Macropus eugenii*, from body measurements. – CSIRO. Division of Wildlife and Ecology. Technical Memorandum 1991. No. 32. Canberra, Australia.

Portmann, A. 1951. Ontogenesetypus und Cerebralisation in der Evolution der Vögel und Säuger. – Rev. Suisse Zool. 54: 427-434.

Pournelle, G.H. 1965. Observations on birth and early development of the spotted hyena. – J. Mammal. 46: 503.

Pournelle, G.H. 1967. Observations on reproductive behaviours and early postnatal development of the proboscis monkey *Nasalis larvatus orientalis* at San Diego Zoo. – Int. Zoo Yearb. 7: 90-92.

Price, M. 1953. The reproductive cycle of the water shrew *Neomys fodiens bicolor* Shaw. – Proc. Zool. Soc. London 128: 599-621.

Prothero, D.R. 1997. The chronological, climatic, and paleogeographic background to North American mammal evolution. – In: Janis, C.M., Scott, K.M. and Jacobs, L.L (eds.). Evolution of Tertiary mammals of North America. Vol. 1: Terrestrial carnivores, ungulates, and ungulate-like mammals. Cambridge University Press. Cambridge.

Pruitt, W.O. 1954. Notes on a litter of young masked shrews. – J. Mammal. 35: 109-110.

Puschmann, W. 2004. Zootierhaltung – Tiere in menschlicher Obhut. 4. Auflage. Verlag Harri Deutsch, Frankfurt a. M.

Rahm, U. 1962. L‘ elevage et la reproduction en captivite de l‘ *Atherurus africanus*. – Mammalia 26: 1-9.

Rahm, U. 1969. Gestation period and litter size of the mole rat, *Tachyoryctes ruandae*. – J. Mammal. 50: 383-384.

Rahn, H. 1982. Comparison of embryonic development in birds and mammals: birth weight, time, and cost. – In: Taylor, C.R., Johansen, K. and Bolis, L. (eds.). A companion to animal physiology. Cambridge University Press. Cambridge.

Ramsay, M.A. and Dunbrack, R.L. 1986. Physiological constraints on life history phenomena: the example of small bear cubs at birth.- Am. Natural. 127: 735-743.

Randall, D., Gannon, B., Runciman, S. and Baudinette, R.V. 1984. Gas transfer by the neonate in the pouch of the tammar wallaby, *Macropus eugenii*. – Persp. Vert. Sci. 3: 423-436.

Rathburn, G.B. 1976. The ecology and social structure of the elephant shrews, *Rhynchocyon chrysopygus* Günther and *Elephantulus rufescens* Peters. PhD. Thesis, University of Nairobi.

Rathburn, G.B. 1979. The social structure and ecology of elephant-shrews. - Advances in Ethology. Supplement 20 to Journal of Comparativ Ethology. Verlag P. Parey. Berlin. Hamburg.

Reeves, J.T. and Leathers, J.E. 1964. Circulatory changes following birth of the calf and the effect of hypoxia. – Circul. Res. 15: 343-354.

Renfree, M.B. 1983. Marsupial reproduction: the choice between placentation and lactation. – In: Finn, C.A. (ed.). Oxford Reviews of Reproductive Biology. 5th ed. Oxford University Press. Oxford.

Renfree, M.B. and Lewis, A.McD. 1996. Cleavage in vivo and in vitro in the Marsupial *Macropus eugenii*. – Reprod. Fertil. Dev. 8: 725-742.

Renfree, M.B. and Tyndale-Biscoe, C.H. 1978. Manipulation of marsupial embryos and pouch young. – In: Daniel, J.C. (ed.). Methodes of Mammalian Reproduction. Academic Press. London.

Renfree, M.B., Pask, A. and Shaw, G. 2001. Sex down under: the differentiation of sexual dimorphisms during marsupial development. – Reprod. Fertil. Dev. 13: 679-690.

Renfree, M.B., Fletcher, T.P., Blandon, D.R., Lewis, P.R., Shaw, G., Gordon, K., Short, R.V., Parer-Cook, E. and Parer, D. 1989. Physiological and behavioural events around the time of birth in macropodid marsupials. – In: Grigg, G, Jarman, P. and Hume, I.D. (eds.). Kangaroos, Wallabies and Rat Kangaroos. Surrey Beatty and Sons Pty. Ltd. Sydney. pp 323-337.

Reynolds, H.C. 1952. Studies on reproduction in the opossum (*Didelphis virginiana virginiana*). – Univ. Calif. Publ. Zool. 52: 223-284.

Ribbons, K.A., Baudinette, R.V. and McMurchie, E.J. 1989. The development of pulmonary surfactant lipids in a neonatal marsupial and the rat. – Resp. Physiol. 75: 1-10.

Richardson, W.B. 1942. Ring-tailed cats (*Bassariscus astutus*): Their growth and development. – J. Mammal. 23: 17-26.

Richardson, W.B. 1943. Wood rats (*Neotoma albigula*): their growth and development. – J. Mamm. 24: 130-143.

Richter, W. von. 1966. Untersuchung über angeborene Verhaltensweisen des Schabrackentapirs und des Flachlandtapirs. – Zool. Beitr. Inst. Univ. Erlangen-Nurenburg. 1*(12)*: 67-159.

Rink, R.D. 1969. Oxygen consumption, body temperature, and brown adipose tissue in the postnatal golden hamster (*Mesocricetus auratus*). – J. Exp. Zool. 170: 117-123.

Robbins, C.T. 1983. Wildlife feeding and nutrition. Academic Press. New York.

Roberts, M.S. and Kessler, D.S. 1979. Reproduction in red pandas, *Ailurus fulgens* (Carnivora: Ailuropodidae). – J. Zool. (London) 188: 235-249.

Romeis, B. 1989. Mikroskopische Technik. 17. Auflage, Verlag Urban und Schwarzenberg, München, Wien, Baltimore.

Romer, J. D. 1974. Milk analysis and weaning in the lesser Malay chevrotain. – Int. Zoo Yearb. 14: 179-180.

Rose, K.D. and Archibald, J.D. 2005. The rise of placental mammals. John Hopkins University Press. Baltimore. London.

Rose, R.W. and Kuswanti, N. 2004. Thyroid function and the development of endothermy in a marsupial, the Tasmanian bettong, *Bettongia gaimardi* (Desmarest 1822). – Gen. Comp. Endocrinol. 136: 17-22.

Rosenthal, M. 1975. The management, behavior and reproduction of the short-eared elephant shrew. M.A. thesis. Northeastern Illinois University.

Rothe, H. 1977. Parturition and related behavior in *Callithrix jacchus* (Ceboidea, Callitrichidae). – In: Kleiman, D.G. (ed.). The biology and conservation of the Callitrichidae. Smithsonian Institution Press. Washington, D.C.

Runciman, S.I.C. 1994. Cardiovascular and respiratory development in the tammar wallaby, *Macropus eugenii.* Diss. Med., Flinders University of South Australia.

Runciman, S.I.C., Baudinette, R.V. and Gannon, B.J. 1996. Postnatal development of the lung parenchyma in a marsupial: The tammar wallaby. – Anat. Record. 244: 193-206.

Runciman, S.I.C., Baudinette, R.V., Gannon, B.J. and Lipsett, J. 1998a. Morphometric estimate of gas-exchange tissue in the new-born tammar wallaby, *Macropus eugenii*. – Resp. Physiol. 111: 177-187.

Runciman, S.I.C., Baudinette, R.V., Gannon, B.J. and Lipsett, J. 1998b. Morphometric analysis of postnatal lung development in the tammar wallaby: Light microscopy. – Resp. Physiol. 112: 325-337.

Runciman, S.I.C., Baudinette, R.V., Gannon, B.J. and Lipsett, J. 1999. Morphometric analysis of postnatal lung development in a marsupial: transmission electron microscopy. – Resp. Physiol. 118: 61-75.

Rudd, C.D. 1994. Sexual behaviour of male and female tammar wallabies (*Macrous eugenii*) at post-partum oestrus. – J. Zool (Lond.). 232: 151-162.

Russell, E.M. 1982. Patterns of parental care and parental investment in marsupials. – Biol. Reviews. 57: 423-486.

Ru-Yung, S. and Jinxiang, Z. 1987. Postnatal development of thermoregulation in the root vole (*Microtus oeconomus*) and the quantitative index of homeothermic ability. – J. therm. Biol. 12 *(4)*: 267-272.

Sabatini, D., Bensch, K. und Barrnett, R. 1963. The preservation of cellular ultrastructure and enzymatic activity by cytochemistry and electron microscopy. – J. Cell Biol. 17: 19-58.

Sadleir, R.M.F.S. 1963. Age estimation by measurement of joeys of the euro *Macropus robustus* Gould in Western Australia. – Austr. J. Zool. 11: 241-249.

Sant' Anna, G. and Mortola, J.P. 2002. Thermal and respiratory control in young rats with altered caloric intake during postnatal development. – Respir. Physiol. Neurobiol. 133: 215-227.

Savage, V.M., Gillooly, J.F., Woodruff, W.H., West, G.B., Allen, A.P., Enquist, B.J. and Brown, J.H. 2004. The predominance of quarter-power scaling in biology. – Funct. Ecol. 18: 257-282.

Scheffer, T.H. 1938. Breeding records of Pacific coast pocket gophers. – J. Mammal. 19: 220-224.

Scheuermann, D., Van Meir, F., Adriaensen, D., Timmermans, J.-P. and De Groodt-Lasseel, Marie H.A. 1988. Development of alveolar septa and formation of alveolar pores during the early postnatal period in the rat lung. – Acta Anat. 131: 249-261.

Schiebler, T.H. and Peiper, U. 1984. Histologie. Lehrbuch der Cytologie, Histologie und mikroskopischen Anatomie des Menschen. Springer Verlag. Berlin, Heidelberg, New York, Tokyo.

Schmidt, C.R. 1973. Breeding season and notes on some other aspects of reproduction in captive camelids. – Int. Zoo Yearb. 13: 387-390.

Schmidt, H.M. 1996. Lungenentwicklung einer physiologischen Frühgeburt (*Monodelphis domestica*). Med. Diss. Georg-August-Universität Göttingen.

Schultz, J., Szdzuy, K. and Zeller, U. 2004. A comparative study of the viscera of metatherian and altricial and precocial eutherian neonates. – Advances in Ethology, Supplements to ethology. 38: 115.

Setchell, P.J. 1970. The development of the thyroid gland of the pouch young of the Tammar wallaby *Macropus eugenii*. Master-thesis. University of Canberra.

Setchell, P.J. 1974. The development of thermoregulation and thyroid function in the marsupial *Macropus eugenii* (Desmarest). – Comp. Biochem. Physiol. A 47: 1115-1121.

Sharman, G.B. 1959. Evolution of marsupials. – Austr. J. Sci. 22.

Sharman, G.B. 1973. Adaptations of marsupial pouch young for extra-uterine existence. – In: Austin, C.R. (ed.). The mammalian fetus in vitro. Chapman and Hall. London.

Sharman, G,B. and Calaby, J.H. 1964. Reproductive behaviour in the red kangaroo, *Megaleia rufa*, in captivity. – C.S.I.R.O. Wildl. Res. 9: 58-85.

Shaw, W.T. 1925. Breeding and development of the Columbian ground squirrel. – J. Mammal. 6: 106-113.

Shaw, G. and Renfree, M.B. 2001. Fetal control of parturition in marsupials. – Reprod. Fertil. Dev. 13: 653-659.

Shield, J. 1966. Oxygen consumption during pouch development of the macropod marsupial *Setonix brachyurus*. – J. Physiol. 187: 257-270.

Shield, J.W. and Woolley, P. 1961. Age estimation and measurements of the pouch young of the quokka (*Setonix brachyurus*). – Austr. J. Zool. 9: 14-23.

Sikes, S.K. 1971. The natural history of the African elephant. Weidenfeld and Nicolson. London.

Singer, D. 1989. Der Winterschlaf als „Naturexperiment" zur Temperatursenkung und Umsatzreduktion bei homöothermen Organismen. Med. Diss. Georg-August-Universität Göttingen.

Singer, D. 1998. Anpassung an Unreife und Sauerstoffmangel in der Neonatalperiode. Vergleichende kalorimetrische Untersuchungen. Med. Habil. Georg-August-Universität Göttingen.

Singer, D. 2001. Anpassung an Unreife und Sauerstoffmangel in der Neonatalperiode. Vergleichende kalorimetrische Untersuchungen. Shaker Verlag, Aachen.

Singer, D. 2004. Metabolic adaptation to hypoxia: cost and benefit of being small. – Respir. Physiol. Neurobiol. 141: 215-228.

Smith, A.G., Smith, D.G. and Funnell, B.M. 1994. Atlas of Mesozoic and Cainozoic coastlines. Cambridge University Press. Cambridge.

Smith, R.E. and Horowitz, B.A. 1969. Brown fat and thermogenesis. – Physiol. Rev. 49: 330-425.

Snyder, J.M. and Magliato, S.A. 1991. An ultrastructural, morphometric analysis of rabbit fetal lung type II cell deifferentiation in Vivo. – Anat. Rec. 229: 73-85.

Snyder, J.M., Rodgers, H.F., O'Brien J.A., Mahli, N., Magliato, S.A. and Durham, P.L. 1992. Glucocorticoid effects on rabbit fetal lung maturation in Vivo: an ultrastructural morphometric study. – Anat. Rec. 232: 133-140.

Sollberger, D.E. 1943. Notes on the breeding habits of the eastern flying squirrel. – J. Mammal. 24: 163-173.

Speakman, J.R. 2005. Body size, energy metabolism and lifespan. – J. Exp. Biol. 208: 1717-1730.

Springer, M.S. 1997. Molecular clocks and the timing of the placental and marsupial relations in relation to the Cretaceous-Tertiary boundary. – J. Mamm. Evol. 4 *(4)*: 285-302.

Springer, M.S., Kirsch, J.A.W. and Case, J.A. 1997. The chronicle of marsupial evolution. – In: Givinish, T.J. and Sytsma (eds.). Molecular Evolution and Adaptive Radiation. Cambridge Univ. Press. Cambridge.

Springer, M.S., Murphy, W.J., Eizirik, E. and O'Brien, S. 2003. Placental mammal diversification and the Cretaceous-Tertiary boundary. - Proc. Natl. Acad. Sci. 100: 1056-1061.

Springer, M.S., Westerman, M. and Kirsch, J.A.W. 1994. Relationships among orders and families of marsupials based on 12S ribosomal DANN sequences and the timing of marsupial radiation. – J. Mamm. Evol. 2: 85-115.

Stanhope, M.J., Waddell, V.G., Madsen, O., de Jong, W., Hedges, S.B., Cleven, G.C., Kao, D. and Springer, M.S. 1998. Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals. – Proc. Natl. Acad. Sci. 95: 9967-9972.

Sterba, O. 1977a. Prenatal development of selected altricial and precocial rodents. – Acta Sc. Nat. Brno. 11 *(11)*: 1-36.

Sterba, O. 1977b. Prenatal development of central european insectivores. – Folia Zool. 26 *(1)*: 27-44.

Stewart, J.H., Rose, R.J. and Barko, A.M. 1984. Respiratory studies in foals from birth to seven days old. – Equine Veter. J. 16: 323-328.

Sullivan, L.C., Orgeig, S. and Daniels, C.B. 2003. The role of extrinsic and intrinsic factors in the evolution of the control of pulmonary surfactant maturation during development in the amniotes. – Physiol.Biochem. Zool. 76 *(3)*: 281-295.

Sundin, U. and Cannon, B. 1980. GDP-binding to the brown fat mitochondria of developing and cold adapted rats. – Comp. Biochem. Physiol. B. 65: 463-471.

Sundin, U., Herron, D. and Cannon, B. 1981. Brown fat thermoregulation in developing hamsters (*Mesocricetus auratus*): a GDP-binding study. – Biol. Neonate. 39: 141-149.

Szalay, F.S. 1993. Metatherian taxon phylogeny: evidence and interpretation from the cranioskeletal system. – In: Szalay, F.S., Novacek, M.J. and McKenna, M.C. (eds.). Mammal Phylogeny, vol.1. Mesozoic Differentiation, Multituberculates, Monotremes, Early Therians, and Marsupials. Springer-Verlag. New York.

Szdzuy, K., Zeller, U. and Renfree, M.B. 2004. Mammalian Evolution: Differences between placental and marsupial offspring. – Advances in Ethology, Supplements to ethology. 38: 118.

Taylor, P.M. 1960. Oxygen consumption in new-born rats. – J. Physiol. 154: 153-168.

Taylor, W.P. 1956. The deer of North America. Stackpole. Harrisburg.

Temple-Smith, P. and Grant, T. 2001. Uncertain breeding: a short history of reproduction in monotremes. – Reprod. Fertil. Dev. 13: 487-497.

Ten Have-Opbroek, A.A.W. 1980. The development of the lung in the mouse. A morphological and immunological study. Diss. University of Leiden.

Ten Have-Opbroek, A.A.W. 1981. The development of the lung in mammals: An analysis of concepts and findings. – Am. J. Anat. 162: 201-219.

Thomas, W. 1975. Observations on captive brockets *Mazame americana* and *M. gouazoubira*. – Int. Zoo Yearb. 15: 77-78.

Thurlbeck, W.M. 1975. Postnatal growth and development of the lung. – Am. Rev. Respir. Dis. 111: 803-844.

Tibben, E.A., Holland, R.A.B. and Tyndale-Biscoe, C.H. 1991. Blood oxygen carriage in the marsupial, tammar wallaby (*Macropus eugenii*), at prenatal and neonatal stages. – Respir. Physiol. 84: 93-104.

Trillmich, F. 2000. Effects of low temperature and photoperiod on reproduction in the female wild guinea pig (*Cavia aperea*). – J. Mamm. 81 *(2)*: 586-594.

Trott, J.F., Simpson, K.J., Moyle, R.L.C., Hearn, C.M., Shaw, G., Nicholas, K.R. and Renfree, M.B. 2003. Maternal regulation of milk composition, milk production, and pouch young development during lactation in the Tammar wallaby (*Macropus eugenii*). – Biol. Reprod. 68: 929-936.

Tyndale-Biscoe, C.H. 2001. Australasian marsupials - to cherish and to hold. – Reprod. Fertil. Dev. 13: 477-485.

Tyndale-Biscoe,C.H. and Renfree,M.B. 1987. Reproductive Physiology of Marsupials. Cambridge University Press, Cambridge.

Tyndale-Biscoe, C.H. and Janssens, P.A. 1988. Introduction. – In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

VandeBerg, J.L. 1999. The laboratory opossum, *Monodelphis domestica*. – In: Poole, T. and English, P. (eds.). The UFAW Handbook on the care and management of laboratory animals. Blackwell Science, Oxford.

Vaughan, T.A., Ryan, J.M. and Czaplewski, N.J. 2000. Mammalogy. 4th edition. Saunders, Orlando.

Vinter, J., Hull, D. and Elphick, M.C. 1982. Onset of thermogenesis in response to cold in newborn mice. – Biol. Neonate. 42: 145-151.

Vogel, P. 1972. Vergleichende Untersuchung zum Ontogenesemodus einheimischer Soriciden (*Crocidura russula, Sorex araneus* und *Neomys fodiens*). – Rev. Suisse Zool. 79: 1201-1332.

Wackernagel, H. 1966. Grant's zebra, *Equus burchelli boehmi* at Basle Zoo: A contribution to breeding biology. – Int. Zoo Yearb. 6: 38-41.

Wadsworth, C.E. 1969. Reproduction and growth of *Eutamias quadrivittatus* in southeastern Utah. – J. Mammal. 50: 256-261.

Waldschmidt, A. and Müller, E.F. 1988. A comparison of postnatal thermal physiology and energetics in an altricial (*Gerbillus perpallidus*) and a precocial (*Acomys cahirinus*) rodent species. – Comp. Biochem. Physiol. A. 90: 169-181.

Walker, M.T. and Gemmell, R.T. 1983. Organogenesis of the pituitary, adrenal, and lung at birth in the wallaby, *Macropus rufogriseus*. - Am. J. Anat. 168: 331-344.

Wandrey, R. 1992. Wale, Delphine und andere Meeressäugetiere. Orbis Verlag. München.

Wang, N.S., Kotas, R.V., Avery, M.E. and Thurlbeck, W.M. 1971. Accelerated appearance of osmiophilic bodies in fetal lungs following steroid injection. – J. Appl. Physiol. 30 *(3)*: 362-365.

Webb, D.R. and McClure, P.A. 1989. Development of heat production in altricial and precocial rodents: implications for the energy allocation hypothesis. – Physiol. Zool. 62: 1293-1315.

Weibel, E.R. 1967. Postnatal growth of the lung and pulmonary gas-exchange capacity. – In: Development of the lung. A Ciba Foundation Symposium. Churchill Ltd. London.

Weibel, E.R. 1970. Functional morphology of the growing lung. – Respir. Suppl. 27: 27-35.

Weibel, E.R. 1980. Design and Structure of the human lung. - In: Fishman, A.P. (ed.). Pulmonary diseases and disorders, Vol.1. Mc Graw Hill, New York.

Weigold, H. 1979. Körpertemperatur, Sauerstoffverbrauch und Herzfrequenz bei *Tupaia belangeri* Wagner, 1841 im Tagesverlauf. – Z. Säugetierkunde 44: 343-353.

Weil, A. 2002. Mammalian Evolution: Upwards and onwards. – Nature. 416: 798-799.

Weir, B.J. 1974. Reproductive characteristics of the hystricomorph rodents. – Symp. Zool. Soc. London. 34: 265-301.

Wemmer, C.M. 1977. Comparative ethology of the large spotted genet (*Genetta tigrina*) and some related viverrids. – Smithsonian Contrib. Zool. 239: 1-93.

Western, D. 1979. Size life history and ecology in mammals. – African J. Ecol. 17: 185-204.

Westheide, W. and Rieger, R. 2004. Spezielle Zoologie. Wirbel- oder Schädeltiere. Spektrum.

White, C.R. and Seymour, R.S. 2003. Mammalian basal metabolic rate is proportional to body mass 2/3. – Proc. Nat. Acad. Sci. 100 *(7)*: 4046-4049.

White, C.R. and Seymour, R.S. 2005. Allometric scaling of mammalian metabolism. – J. Exp. Biol. 208: 1611-1619.

Whittaker, D. 1999. Hamsters. – In: Poole, T. and English, P. (eds.). The UFAW Handbook on the care and management of laboratory animals. Blackwell Science, Oxford.

Wilkes, G.E. and Janssens, P.A. 1988. The development of renal function. In: Tyndale-Biscoe, C.H. and Janssens, P.A. (eds.). The developing marsupial. Models for Biomedical Research. Springer-Verlag, Berlin, Heidelberg.

Williams, L. 1967. Breeding Humboldt's wooley monkey *Lagothrix lagotricha* at Murrayton Wooley Monkey Sanctuary. – Int. Zoo Yearb. 6: 86-89.

Wilmer, P., Stone, G. and Johnston, I. 2000. Environmental Physiology of Animals. Blackwell Science. Oxford. London.Edinburgh.

Wilson, D.E. and Reeder, D.M. 1993. Mammal species of the world: A taxonomic and geographic reference. Smithsonian Inst. Press, Washington D.C.

Wimsatt, W.A. 1960. An analysis of parturition in Chiroptera, including new observations on *Myotis lucifugus*. – J. Mammal. 41: 183-200.

Winkler, G.C. and Cheville, N.F. 1984. The neonatal porcine lung: Ultrastructural morphology and postnatal development of the terminal airways and alveolar region. – Anat. Rec. 210: 303-313.

Winkler, G.C. and Cheville, N.F. 1985. Morphometry of postnatal development in the porcine lung. – Anat. Rec. 211: 427-433.

Withers, P.C. and Jarvis, J.U.M. 1980. The effect of huddling on thermoregulation and oxygen consumption for the naked mole-rat. – Comp. Biochem. Physiol. A. 66: 215-219.

Witschi, E. 1962. Growth including reproduction and morphological development. VII. Prenatal vertebrate development. Fed. Am. Soc. Exp. Biol. Washington.

Wollburg, I. 1957. Über die Entwicklung der Thermoregulation bei Jungsäugern. – Zool. Anz., Suppl. 20: 79-85.

Wood, J.E. 1949. Reproductive patterns of the pocket gopher (*Geomys breviceps brazensis*). - J. Mammal. 30: 36-44.

Woodburne, M.O. and Case, J.A. 1996. Dispersal, vicariance, and the late Cretaceous to early Tertiary land mammal biogeography from South America to Australia. – J. Mammal. Evol. 3 *(2)*: 121-161.

Wright, N.G., Brown, R.M.H., McCandlish, I.A.P., Thompson, H. and Cornwell, H.J.C. 1983. Patterns of cilia formation in the lower respiratory tract of the dog: a scanning electron microscopic study. – Res. Vet. Sci. 34: 340-346.

Yates, T.L. 1984. Insectivores, elephant shrews, tree shrews, and dermopterans. – In: Anderson, S. and Jones, J.K. (eds.). Orders and Families of Recent Mammals of the World. John Wiley & Sons, New York.

Zeller, U. 1983. Zur Ontogenese und Morphologie des Craniums von *Tupaia belangeri* (Tupaiidae, Mammalia). Diss. Med., Georg August-Universität, Göttingen.

Zeller, U. 1986a. Ontogeny and cranial morphology of the tympanic region of the Tupaiidae, with special reference to Ptilocercus. - Folia Primatol. 47: 61-80.

Zeller; U. 1986b. The systematic relations of tree shrews: Evidence from skull morphogenesis. – In: Else, J.G. and Lee, P.C. (eds.). Primate Evolution. Cambridge University Press, Cambridge.

Zeller, U. 1999. Mammalian Reproduction: Origin and Evolutionary Transformations. – Zool. Anz. 238: 117-130.

Zeller, U. and Freyer, C. 2001. Early ontogeny and placentation of the gray short-tailed opossum, *Monodelphis domestica* (Didelphidae: Marsupialia): contribution to the reconstruction of the marsupial morphotype. – J. Zool. Syst. Evol. Research. 39: 137-158.

Zeller, U., Freyer, C. and Neumann, C. 2001. Mammalian Reproduction: Origin and evolutionary transformations, with special reference to the K/T-boundary. – Mitt. Mus. Nat.kd. Berl., Zool. Reihe. 77 *(2)*: 245-246.

Zeller, U., Freyer, C., Szdzuy, K., Schultz, J. 2004. Stem species patterns of mammalian reproduction. - J. Morph. 260: 342.

Zeltner, T.B. and Burri, P.H. 1987. The postnatal development and growth of the human lung. II. Morphology. – Respir. Physiol. 67: 269-282.

Zeltner, T.B., Caduff, J.H., Gehr, P., Pfenninger, J. and Burri, P.H. 1987. The postnatal development and growth of the human lung. I. Morphometry. – Respi. Physiol. 67: 247-267.

Zullinger, E.M., Ricklefs, R.E., Redford, K.H. and Mace, G.M. 1984. Fitting sigmoidal equations to mammalian growth curves. – J. Mammal. 65: 607-636.

Appendix

- **Table I** Survey of the histological and ultrastructural investigations of the species examined
- **Table II.** Body weights during the postnatal development of the six species examined
- **Table III** Reproduction and Development for selected mammalian species

Tab. I: Survey of the histological and ultrastructural investigations of the species examined

Species	Age stage	No.	Methode	Fixation	Embedding	Thickness	Staining	Source	Comments
Prototheria									
Monotremata									
Ornithorhynchus anatinus	pouch young	201969	LM		Celloidin	10 µm	AZ	CH	torso
Tachyglossus aculeatus	pouch young	75	LM		Paraffin	12 µm	AZ	CH	torso
Theria									
Metatheria									
Monodelphis domestica	neonate	337	LM	inst./B.	paraffin	10 µm	AZ	CZ	torso
		86	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			right lung
		137	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
		138	SEM						lung
	4 dpn		LM	B.	paraffin	10 µm	AZ	CZ	torso
	5 dpn	140/A	LM	inst./B.	Kulzer	2 µm	HE	CZ	lungs and trachea
		268	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
		269	SEM						lung
	7 dpn	1102	SEM	perf. r., inst./gl./pfa.					left and right lung
	8 dpn	89	LM	B.	paraffin	7 µm	AZ	CZ	torso
	12 dpn	112	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
	14 dpn	92	LM	B.	paraffin	10 µm	AZ	CZ	torso
		1104	SEM	perf. r., inst./gl./pfa.					left and right lung
	21 dpn	1082	LM	B.	paraffin	10 µm	TC		torso
		1081	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
		1080	SEM	perf. r., inst./gl./pfa.					left and right lung
	28 dpn	1059	LM	B.	paraffin	10 µm	AZ		torso
		1058	SEM	perf. r., inst./gl./pfa.					left and right lung
		150	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
	41 dpn	131	LM	perf./B.	paraffin	10 µm	HE	CZ	lungs and heart
	56 dpn	1061	LM	perf./B.	paraffin	10 µm	TC		lungs and heart

Species	Age stage	No.	Me-thode	Fixation	Embed-ding	Thick-ness	Stain-ing	Sour-ce	Comments
		1060	SEM	perf. r., inst./gl./pfa.					right lung
		1060	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left lung
	3 months (adult)	105	LM	perf./B.	paraffin	10 µm	HE	CZ	left lung
		291	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
		1030	SEM	perf. r., inst./gl./pfa.					left and right lung
	adult	1830	LM	perf./B.	Kulzer	2 µm	HE	CZ	left lung
		273	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
Macropus eugenii	neonate	2315	LM	-	paraffin	8 µm	TC	CR	left and right lung
	5 dpn	2080	LM	-	paraffin	8 µm	TC	CR	left and right lung
	10 dpn	2320	LM	-	paraffin	8 µm	TC	CR	left and right lung
	11 dpn	0821	LM	pfa.	paraffin	8 µm	TC	CR	left and right lung
	24 dpn	2110	LM	pfa.	paraffin	8 µm	TC	CR	left and right lung
	31 dpn	1659	LM	pfa.	paraffin	8 µm	TC	CR	left and right lung
	42 dpn	5	LM	pfa.	paraffin	8 µm	TC	CR	left and right lung
	55 dpn	1663	LM	pfa.	paraffin	8 µm	TC	CR	left and right lung
	65 dpn	3760	LM	pfa.	paraffin	10 µm	TC	CR	left and right lung
	71 dpn	3438	LM	pfa.	paraffin	8 µm	TC	CR	left and right lung
	75 dpn	3390	LM	pfa.	paraffin	8 µm	TC	CR	right lung
	85 dpn	2286	LM	pfa.	paraffin	8 µm	TC	CR	right lung
	98 dpn	2256	LM	pfa.	paraffin	8 µm	TC	CR	right lung
	142 dpn	KIPY	LM	pfa.	paraffin	8 µm	TC	CR	right lung
Eutheria									
Mesocricetus auratus	neonate	14	LM	B.	paraffin	10 µm	HE	CZ	torso
		22	LM	B.	paraffin	10 µm	HE	CZ	torso
		40	SEM	perf. r., inst./gl./pfa.					left and right lung
		41	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung

Species	Age stage	No.	Me-thode	Fixation	Embed-ding	Thick-ness	Stain-ing	Sour-ce	Comments
	2 dpn	50	LM	B.	paraffin	10 µm	HE		torso
		49	SEM	perf. r., inst./gl./pfa.					left and right lung
		49	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
	4 dpn	43	LM	B.	paraffin	10 µm	HE		torso
		42	SEM	perf. r., inst./gl./pfa.					left lung
		42	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			right lung
	7 dpn	44	LM	B.	paraffin	12 µm	HE		torso
		53	SEM	perf. r., inst./gl./pfa.					left and right lung
		53	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
	11 dpn	45	LM	perf. r., inst./B.	paraffin	10 µm	HE		lungs and heart
	14 dpn	46	LM	perf. r., inst./B.	paraffin	10 µm	HE		lungs and heart
	adult	35	LM	perf. r., inst./B.	paraffin	10 µm	TC		left lung
		35	SEM	perf. r., inst./gl./pfa.					right lung
		35	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			right lung
Suncus murinus	neonate	11	LM	B.	paraffin	10 µm	HE	CZ	torso
		34	SEM	perf. r., inst./gl./pfa.					right lung
		34	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			right lung
	4 dpn	26	LM	B.	paraffin	10 µm	HE		torso
		29	SEM	perf. r., inst./gl./pfa.					left and right lung
		29	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			right lung
	7 dpn	-	LM	B.	paraffin	10 µm	HE	CZ	lungs and heart
		36	SEM	perf. r., inst./gl./pfa.					left and right lung
		36	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left and right lung
	11 dpn	55	LM	perf. re., inst./B.	paraffin	10 µm	TC		lungs and heart
	14 dpn	47	LM	perf. re., inst./B.	paraffin	10 µm	TC		lungs and heart

Species	Age stage	No.	Me-thode	Fixation	Embed-ding	Thickn ess	Stain-ing	Sour-ce	Comments
	adult	18	LM	B.	paraffin	10 µm	HE		lungs and heart
		14	LM	B.	paraffin	10 µm	HE		left lung
		14	SEM	gl./pfa.					right lung
		14	TEM	gl./pfa.	Araldite	70 nm			right lung
Tupaia belangeri	neonate	1294 3	LM	B.	paraffin	8 µm	TC		lungs and heart; still-birth
		1307 5	LM	B.	paraffin	8 µm	TC		right lung
		1307 5	SEM	gl./pfa.					left lung
		1307 5	TEM	gl./pfa.	Araldite	70 nm			left lung
	4 dpn	1307 2	LM	B.	paraffin	8 µm	TC		right lung
		1307 2	SEM	gl./pfa.					left lung
		1307 2	TEM	gl./pfa.	Araldite	70 nm			left lung
	7 dpn	1307 3	LM	B.	paraffin	8 µm	TC		right lung
		1307 3	SEM	gl./pfa.					left lung
		1307 3	TEM	gl./pfa.	Araldite	70 nm			left lung
Cavia aperea	neonate	7	LM	B.	paraffin	10 µm	TC		left lung
		7	SEM	gl./pfa.					right lung
		7	TEM	gl./pfa.	Araldite	70 nm			right lung
		23	LM	B.	paraffin	8 µm	TC		Left and right lung; stillbirth
	4 dpn	8	LM	B.	paraffin	10 µm	TC		left lung
		8	SEM	gl./pfa.					right lung
		8	TEM	gl./pfa.	Araldite	70 nm			right lung
	adult	26	LM	B.	paraffin	10 µm	TC		right lung
		26	SEM	perf. r., inst./gl./pfa.					left lung
		26	TEM	perf. r., inst./gl./pfa.	Araldite	70 nm			left lung
Macroscelides proboscideus	neonate	9	LM	B.	paraffin	12 µm	HE, AZ		torso
	adult	4	LM	B.	paraffin	10 µm	TC		Left and right lung

inst.	fixation by instillation via trachea
perf.	fixation by perfusion
perf,r.	fixation by perfusion via the infundibulum of the right ventricle
B.	Bouin’s blend
gl.	glutaraldehyde
pfa.	paraformaldehyde
Kulzer	synthetic resin
AZ	Azan stain
HE	Haematoxylin eosin stain
TC	Trichrom stain (according to Masson and Goldner)
CZ	collection of Prof. Dr. U. Zeller
CH	collection of Hubrecht Laboratory
CR	collection of Prof. Dr. M.B. Renfree
dpn	dies post natum

Tab. II: Body weights during the postnatal development of the six species examined

	Body weight (mean ± standard derivation (number of animals))					
Age (days)	*Monodelphis domestica*	*Macropus eugenii*	*Tupaia belangeri*	*Suncus murinus*	*Mesocricetus auratus*	*Cavia aperea*
0	0.09 ± 0.01 (N=8)	0.37	18.30 ± 0.85 (N=9)	2.79 ± 0.50 (N=11)	2.10 ± 0.39 (N=22)	60.51 ± 5.34 (N=12)
1	0.13 ± 0.01 (N=5)	-	17.61 ± 0,59 (N=9)	3.50 ± 0.77 (N=9)	3.20 ± 0.45 (N=17)	61.01 ± 5.71 (N=12)
2	0.14 (N=1)	-	24.55 ± 2.10 (N=9)	4.79 ± 1.20 (N=9)	3.56 ± 0.40 (N=15)	62.86 ± 6.71 (N=12)
3	0.17 ± 0.02 (N=6)	-	23.29 ± 1.70 (N=9)	6.33 ± 1.36 (N=9)	4.05 ± 0.09 (N=7)	66.68 ± 7.60 (N=12)
4	0.21 ± 0.06 (N=2)	-	30.19 ± 3.53 (N=9)	7.78 ± 1.78 (N=9)	5.03 ± 0.44 (N=14)	70.78 ± 7.82 (N=12)
5	0.23 ± 0.03 (N=7)	0.87	28.53 ± 3.62 (N=9)	10.04 ± 2.13 (N=9)	5.70 ± 0.45 (N=12)	76.67 ± 9.13 (N=12)
6	0.27 ± 0.01 (N=4)	-	35.23 ± 3.49 (N=9)	11.70 ± 2.11 (N=9)	6.44 ± 0.80 (N=12)	81.27 ± 10.0 (N=12)
7	0.42 ± 0.10 (N=2)	-	32.52 ± 3.82 (N=9)	13.24 ± 2.39 (N=9)	7.08 ± 0.88 (N=14)	86.93 ± 10.21 (N=12)
8	-	-	41.79 ±4.17 (N=9)	15.21 ± 2.19 (N=4)	7.58 ± 1.14 (N=12)	91.72 ± 9.92 (N=12=
9	0.44 ± 0.04 (N=9)	-	39.20 ± 5.73 (N=9)	16.92 ±1.93 (N=9)	8.79 ± 1.03 (N=12)	97.02 ± 12.88 (N=12)
10	-	1.29	50.25 ± 5.04 (N=9)	18.48 ± 2.14 (N=9)	10.28 ± 1.19 (N=9)	103.25 ± 11.36 (N=12)
11	0.64 ± 0.02 (N=2)	-	47.77 ± 4.65 (N=9)	20.80 ± 2.40 (N=9)	11.61 ± 2.16 (N=10)	107.91 ± 12.66 (N=11)
12	0.71 ± 0.06 (N=4)	1.41	60.46 ± 7.81 (N=9)	-	-	-
13	0.89 ± 0.03 (N=4)	-	54.72 ± 6.93 (N=9)	-	-	-
14	0.90 ± 0 (N=9)	-	67.86 ± 6.52 (N=9)	24.61 ± 2.73 (N=9)	15.93 ± 2.44 (N=10)	125.92 ± 13.28 (N=11)
15	1.25 ± 0.25 (N=3)	-	63.79 ± 5.13 (N=9)	-	-	-
16	-	-	76.66 ± 6.14 (N=9)	-	-	-
17	1.77 (N=1)	-	72.76 ± 6.18 (N=9)	-	-	-
18	-	-	83.08 ± 6.17 (N=9)	-	-	-
19	1.80 (N=1)	3.13	79.89 ± 4.94 (N=9)	-	-	-
20	1.96 (N=1)	-	89.05 ± 7.56 (N=9)	-	-	-
21	2.06 ± 0.19 (N=9)	-	86.38 ± 7.22 (N=9)	36.69 ± 3.09 (N=9)	37.22 ± 4.02 (N=9)	163.56 ± 16.21 (N=11)

Age (days)	Body weight (mean ± standard derivation (number of animals))					
	Monodelphis domestica	*Macropus eugenii*	*Tupaia belangeri*	*Suncus murinus*	*Mesocricetus auratus*	*Cavia aperea*
22	-	-	101.42 ± 10.36 (N=6)	-	-	-
23	-	-	95.97 ± 8.89 (N=6)	-	-	-
24	3.00 (N=1)	-	109.57 ± 13.98 (N=6)	-	-	-
25	4.00 (N=1)	-	106.40 ± 12.73 (N=6)	-	-	-
26	4.00 ± 0 (N=3)	-	119.17 ± 13.93 (N=6)	-	-	-
27	-	-	115.66 ± 16.15 (N=4)	-	-	-
28	4.03 ± 0.13 (N=9)	-	121.63 ± 15.67 (N=4)	48.77 ± 9.35 (N=9)	59.62 ± 5.56 (N=10)	200.0 (N=1)
29	-	-	120.20 ± 13.28 (N=4)	-	-	-
30	-	-	123.67 ± 12.63 (N=4)	-	-	-
31	5.00 ± 0 (N=2)	-	123.22 ± 19.34 (N=4)	-	-	-
32	-	-	128.77 ± 15.75 (N=4)	-	-	-
33	-	5.58	128.60 ± 19.52 (N=4)	-	-	-
34	-	-	130.77 ± 24.62 (N=4)	-	-	-
35	5.68 ± 0.30 (N=14)	-	133.98 ± 25.75 (N=4)	-	66.21 ± 5.33 (N=3)	226.0 (N=1)
36	-	-	137.88 ± 23.59 (N=4)	-	-	-
37	-	-	141.43 ± 23.62 (N=4)	-	-	-
39	-	10.47	-	-	-	-
41	9.00 (N=1)	10.55	-	-	-	-
42	9.42 ± 0.80 (N=14)	-	-	-	-	254.0 (N=1)
47	-	13.00	-	-	-	-
49	13.48 ± 1.10 (N=15)	-	-	-	-	282.0 (N=1)
56	19.63 ± 1.37 (N=14)	-	-	-	-	328.0 (N=1)
63	27.50 ± 1.79 (N=8)	-	-	-	83.42 ± 7.56 (N=4)	370.0 (N=1)
65	-	27.55	-	-	-	-
71	-	32.60	-	-	-	-

Age (days)	Body weight (mean ± standard derivation (number of animals))					
	Monodelphis domestica	*Macropus eugenii*	*Tupaia belangeri*	*Suncus murinus*	*Mesocricetus auratus*	*Cavia aperea*
87	-	45.42	-	-	-	-
95	-	52.95	-	-	-	-
104	-	70.33	-	-	-	-
118	-	84.42	-	-	-	-
123	-	91.94	-	-	-	-
140	-	97.65	-	-	-	-
Adult	71.95 ± 14.18 (N=11)	-	186.00*	51.26 ± 10.5 (N=18)	95.47 ± 9.42 (N=10)	

* Weigold, 1979

Tab.III: Reproduction and Development for selected mammalian species (Based on Eisenberg (1983) and Puschmann (2004); Systematics after Westheide and Rieger (2004)).

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters / Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Monotremata												
Ornithorhynchus anatinus	1,200	15-21 (10)*	1.3	1	120		77	< 105			24	Fleay, 1944
Tachyglossus aculeatus	4,200	21-23 (10)*	1	1	140	0.3-0.4	75	30-40		50-60	12	Griffiths, 1968
Zaglossus bruijnii		22-30 (10)*	1		180-210		70	30-40		50-60	12	Puschmann, 2004
Marsupialia												
Didelphoidea												
Didelphidae												
Caluromys philander	285-340		4.1		100-125					75-80		Atramentovicz, 1982
Didelphis marsupialis	660-890	12-13	7	2-3	100	0.16-0.21	58-72			70	5-8	Collins, 1973
Didelphis paraguayensis		12-13	6-25	2-3	70		42-56				5-8	Puschmann, 2004
Didelphis virginiana	1000-4000	13	3-13	2-3	110	0.13	58	60	48	70-80	5	Tyndale-Biscoe & Renfree, 1987
Lutreolina crassicaudata		12-13	6-25		70		42-56					Puschmann, 2004
Marmosa robinsoni	37.5	14	11	1-2	65	0.18	39-40		20	35-40	3	Collins, 1973
Monodelphis domestica	60-100	14	3-14 (7.3)	2	49	0.1	28-35	28	14		182 d	Kraus & Fadem, 1987
Philander opossum	250-400	90	4-6	1	90							Tyndale-Biscoe & Renfree, 1987
Dasyuroidea												
Myrmecobiidae												
Myrmecobius fasciatus	500		2.4		180					160		Tyndale-Biscoe & Renfree, 1987
	* gestation period is time from copulation until egg-laying, time until hatching in brackets											

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Dasyuridae												
Antechinomys laniger	63	80	5	2-3	80		50		48	40	5-6	Tyndale-Biscoe & Renfree, 1987
Antechinomys spenceri	24.2	30	2-10	1								Puschmann, 2004
Antechinus flavipes	30		12		90-120	0.016			42			Tyndale-Biscoe & Renfree, 1987
Antechinus stuartii	37	27	6-10	1-2	90	0.018	62			75	9	Collins, 1973
Antechinus swainsonii	50		8-10		90-95				33-43			Tyndale-Biscoe & Renfree, 1987
Dasycercus cristicauda	60-130		6		100-120				55-60	88	12	Tyndale-Biscoe & Renfree, 1987
Dasyuroides byrnei	90-150	35-36	2-6 (4.8)	1	100-120		76	76	56	70-78	12	Tyndale-Biscoe & Renfree, 1987
Dasyurus hallucatus	300-500		6.4		125		70-78		60-70		10-11	Tyndale-Biscoe & Renfree, 1987
Dasyurus viverrinus	1,300	8-14 (5.8)	6	1	135	0.012	63	70	49-56	91	10	Collins, 1973
Dasyurus maculatus	2000-4000		5		150				49	96	12	Tyndale-Biscoe & Renfree, 1987
Ningauri ridei	8-10	13-21	6-7		91	0.005			42-44	42		Tyndale-Biscoe & Renfree, 1987
Phascogale tapoatafa	140-180	30	1-8	1	120				54		7.5	Tyndale-Biscoe & Renfree, 1987
Planigale gilesi	6-10		7		65-70				37	37		Tyndale-Biscoe & Renfree, 1987
Planigale ingrami	6-9		4-12		90				35-40			Tyndale-Biscoe & Renfree, 1987
Planigale maculatus	9-16		4-12		70				28	45	10	Tyndale-Biscoe & Renfree, 1987

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Sarcophilus harrisii	6700-12000	31	1-4 (2.9)	1	140	0.018	87-93		90-105	105	24	Collins, 1973; Tyndale-Biscoe & Renfree, 1987
Sminthopsis crassicaudata	12-18	13-16	10	1-2	70	0.010	49-50		43	59-63	4-5 5-6	Collins, 1973; Tyndale-Biscoe & Renfree, 1987
Sminthopsis macroura	16-24	12.5	1-8		70	0.010			40		4 7	Tyndale-Biscoe & Renfree, 1987
Sminthopsis murina	21		4-10		65				35			Tyndale-Biscoe & Renfree, 1987
Perameloidea												
Peramelidae												
Isoodon macrourus	1130	15	4		63-70	0.18	45	49	30	50	102 d	Collins, 1973
Isoodon obesculus	766		2.8		58	0.35				53	4 6	Tyndale-Biscoe & Renfree, 1987
Macrotis lagotis	800-1100	12.5	2	1	60	0.2				75	3	Tyndale-Biscoe & Renfree, 1987
Perameles gunni	816	11	2.3	4	60	0.25	44-46		32	48-53	3 4	Collins, 1973
Perameles nasuta	860	12-13	2-3		75-80	0.237	44-48		30	50-54	4 5	Puschmann, 2004
Tarsipedoidea												
Tarsipedidae												
Tarsipes rostratus	10-12		2.4		90	0.003-0.006				63-70	6	Tyndale-Biscoe & Renfree, 1987
Phalangeroidea												
Petauridae												
Gymnobelideus leadbeateri	122-133	< 20	1.6		120					87	12	Tyndale-Biscoe & Renfree, 1987

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Petaurus australis	450-700		1	1	180-240					100	24 18	Tyndale-Biscoe & Renfree, 1987
Petaurus breviceps	150-200	16	1-3 (1.6)	2	120	0.194	80		40	70-74	8-15 12	Collins, 1973; Tyndale-Biscoe & Renfree, 1987
Petaurus norfolcensis	220		1-2	1	120							Tyndale-Biscoe & Renfree, 1987
Phalangeridae												
Trichosurus caninus	2500-4500	16.2	1		275				112	175-200	22-36 36	Tyndale-Biscoe & Renfree, 1987
Trichosurus vulpecula	1500-3500	18	1.4	2	230	0.2-0.32	100-110	117	94	140-150	12-24 24	Collins, 1973
Acrobatidae												
Acrobates pygmaeus	11-17		2	2	90-95		70			50	6-8	Tyndale-Biscoe & Renfree, 1987
Burramyridae												
Burramys parvus	40		4		70-75					33-37	12	Tyndale-Biscoe & Renfree, 1987
Cercartetus concinnus	50		4-5		50	0.012			< 25	30	12	Tyndale-Biscoe & Renfree, 1987
Cercartetus nanus	24		4-5		50-60					42	5	Tyndale-Biscoe & Renfree, 1987
Petauroides volans	1000-1250		1	1	240	0.273			75	150	24	Tyndale-Biscoe & Renfree, 1987
Pseudocheirus peregrinus	700-1000	12-50	2	1	160	0.3	91		42-49	120	12	Tyndale-Biscoe & Renfree, 1987

	Reproduction					Development							
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters / Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀	♂	References
Macropodoidea													
Potoroidae													
Aepyprymnus rufescens	1000-3500	21-30	1		155				87	114	10	12	Tyndale-Biscoe & Renfree, 1987
Pseudocheiridae													
Bettongia gaimardi	1800	21.1	1		160	0.30		84		109	8-11		Tyndale-Biscoe & Renfree, 1987
Bettongia lesueur	1100	21	1		165	0.38	83		61	115	7	14	Collins, 1973
Bettongia penicillata	1000-1600	21	1		130	0.29				100	10		Tyndale-Biscoe & Renfree, 1987
Potorous apicalis	1360	33	1			0.33	94						Collins, 1973
Potorous tridactylus	660-1000	38	1	2	120	0.33	94	90	70	130	12		Collins, 1973
Macropodidae													
Macropus agilis	12000	29.4	1		328	0.63				219	12	14	Tyndale-Biscoe & Renfree, 1987
Macropus eugenii	5000	29.3	1	1	270	0.37	140	180	105	250	8	24	Tyndale-Biscoe & Renfree, 1987
Macropus fuliginosus	27600	30.6	1		540	0.828				310	14	31	Tyndale-Biscoe & Renfree, 1987
Macropus giganteus	27600	36.4	1	1	540	0.74	163-181			319	18	48	Tyndale-Biscoe & Renfree, 1987
Macropus parma	3500	34.5	1		300	0.51			120	212	16	22	Tyndale-Biscoe & Renfree, 1987
Macropus parryi	14000	36.3	1		420					275	24		Tyndale-Biscoe & Renfree, 1987
Macropus robustus	16000	31-33	1		380					256	27		Tyndale-Biscoe & Renfree, 1987

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Macropus rufogriseus	14000	29.4	1		360	0.45	100	100-125	70	270	11 13	Tyndale-Biscoe & Renfree, 1987 ; Loudon et al.,1985
Macropus rufus	27300	33.2	1	1	360	0.817	144		70	235	14-20 24-36	Collins, 1973; Tyndale-Biscoe & Renfree, 1987
Peradorcas concinna	1400		1		360					180	12-24	Tyndale-Biscoe & Renfree, 1987
Petrogale penicillata	4000	31	1		290				127	204	18 20	Tyndale-Biscoe & Renfree, 1987
Setonix brachyurus	2750	27	1		240	0.35	140	145	87	190	9-12 13	Tyndale-Biscoe & Renfree, 1987
Thylogale billardierii	3900	30	1		300	0.4				200	14	Tyndale-Biscoe & Renfree, 1987
Thylogale thetis	3900		1		210					181	26 20	Tyndale-Biscoe & Renfree, 1987
Wallabia bicolor	11500	35	1	1		0.61				256	15	Tyndale-Biscoe & Renfree, 1987
Vombatiformes												
Phascolarctidae												
Phascolarctos cinereus	4500-7900	35	1		360-380					240-270	36	Tyndale-Biscoe & Renfree, 1987
Vombatidae												
Lasiorhinus latifrons	25000	21-22	1		400	0.5				300		Tyndale-Biscoe & Renfree, 1987
Vombatus ursinus	26000	21-22	1	1	400	0.5				150	24	Tyndale-Biscoe & Renfree, 1987

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Placentalia												
XENARTHRA												
Xenarthra												
Dasypodidae												
Chaetophractus villosus	1800	60	2	1		145	32				12	Encke, 1965
Dasypus novemcinctus	4000	120	4	1	42-49	40-50	12-18				12	Block, 1974
Euphractus sexcinctus	4900	60-65	1-3		28-35	95-115	22-25				12	Gucwinska, 1971
Euphractus villosus		60-65	1-2		49-56	100-145	16-30				9	Puschmann, 2004
Tolypeutes matacus		60-65	1			85	21-28				12	Meritt, 1976
Bradypodidae												
Bradypus cuculliger		170-175♠	1		365	300-400	0	0			2.5-3 y	Puschmann, 2004
Bradypus torquatus		170-175♠	1		365	300-400	0	0			2.5-3 y	Puschmann, 2004
Bradypus variegatus	4000-4500	170-180♠	1	1	10	230-250	0	0			2.5-3 y	Montgomery & Sunquist, 1978
Choleopus didactylus		235♠	1		75	300-400	0	0			2.5-3 y	Puschmann, 2004
Choleopus hoffmanni	9000	332		0.5	20	340-400	0	0			3 4-5 y	NZP records
Myrmcophagidae												
Tamandua tetradactyla	3500-7000	130-150	1	1.7	180	225-400						NZP records
Myrmecophaga tridactyla	22000-39000	190	1	1.34	180	1.587	6				24	Bickel, Murdock & Smith, 1976
AFROTHERIA												
Afrosoricida												
Tenrecidae												
	♠ including delayed implantation											

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Microgale dobsoni	34-35	62-64	1-2		28-30	4	18-22				22	Eisenberg, 1975
Microgale talazaci	39-61	58-63	1-3		30	3.6	18				22	Eisenberg, 1975
Echinops telfairi	180	62-65	1-10 (5.8)		30	6	7-9				6	Eisenberg, 1975
Hemicentetes nigriceps	90-150	55	2-4	2	20	6.3	8				30-35 d	Eisenberg, 1975
Hemicentetes semispinosus	125-280	57-63	2-11	2	20	6.5	8-10				35-40 d	Eisenberg, 1975
Setifer setosus	180-270	65-69	1-5	1	35-38	24.7	13				6	Eisenberg, 1975
Tenrec ecaudatus	900	57-63	1-3	1-2	29	25	9-14				6	Eisenberg, 1975
Macroscelidea												
Macroscelididae												
Elephantulus brachyrhynchus	45		2	5-6		11-12	0	0			50 d	Rathbun, 1976; Mills & Hes, 1999
Elephantulus edwardii	50		1-2			11-12	0	0			50 d	Mills & Hes, 1999
Elephantulus intufi	50		2			11-12	0	0			50 d	Mills & Hes, 1999
Elephantulus myurus	60	56	1-2			11-12	0	0			35-42 d	Mills & Hes, 1999
Elephantulus rufescens	58	56	1-2	4-5	22	10	0	0			50 d	Rathbun, 1976
Elephantulus rupestris	65	56	1-2			11-12	0	0			50 d	Mills & Hes, 1999
Petrodomus tetradactylus	210		1			30.5	0	0				Rathbun, 1976
Macroscelides proboscideus	45	61	1-2		7	6-8	0	0			35-42 d	Rosenthal, 1975; Mills & Hes, 1999
Rhynchocyon chrysopygus	450	42	1	4-5	14	80	0	0				Rathbun, 1976
Tubulidentata												
Orycteropus afer	60000	239-268	1		112	1870	0				< 3y	Melton, 1976
Hyracoidea												

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Dendrohyrax arboreus		210-240	1-2		90	300-350	0	0			17	Puschmann, 2004
Heterohyrax brucei		210-240	2-4		90	160-200	0	0			17	Puschmann, 2004
Procavia capensis	17000	225	3-4		90	170-240	0	0			24	Mendelsohn, 1965
Procavia habessinica		226-237	2-3		90	170-240	0	0			17	Puschmann, 2004
Sirenia												
Dugongidae												
Dugong dugong	230000	365	1-2		390-420	25000-40000	0	-			3-5 y	Puschmann, 2004
Trichechidae												
Trichechus inunguis	350000	365	1-2		365-540	8000-10000	0	-			3-5 y	Puschmann, 2004
Trichechus manatus		385-400	1	0.3	540	11000-27000	0	-			3-4 y	Hartman, 1971
Trichechus senegalensis	1,600,000	365	1-2		365-540		0	-			3-5 y	Puschmann, 2004
Proboscidea												
Elephas maximus	2,730,000	628	1		365-730	90,900	0	0			9-10 10-12 y	McKay, 1973
Loxodonta africana	2,766,000	660	1		365-730		0	0			9-10 10-12 y	Sikes, 1971
EUARCHONTOGLIRES												
EUARCHONTA												
Primates												
Prosimiae												
Cheirogaleidae												

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Cheirogaleus medius		61-70	2			18	0	0			10-14	Puschmann, 2004
Mirza coquereli		95-100	1-2		119-140	12	0	0			9	Puschmann, 2004
Microcebus murinus	70	60	2		45	10	2	0			7-10	Bourliere et al., 1961
Lemuridae												
Eulemur coronatus		123-126	1-2			55-70	0	0				Puschmann, 2004
Eulemur macaco		120-129	1-2		140-182	55-80	0	0			18	Puschmann, 2004
Eulemur mongoz		126-130	1-2		140-182	41-60	0	0			18	Puschmann, 2004
Hapalemur griseus		140-150	1-2		140-175	40-55	0	0			18	Puschmann, 2004
Lemur catta	2290	127.5	1		168-182	88.2	0	0			18	Leutenegger, 1973
Lemur fulvus	1924	127.5	1		154-182	83.5	0	0			18	Leutenegger, 1973
Varecia variegata		98-106	2		140-196	75-140	0	0			18-20 24-33	Puschmann, 2004
Megaladapidae												
Lepilemur dorsalis		135	1		112		0	0			18	Puschmann, 2004
Lepilemur edwardsi		135	1		112		0	0			18	Puschmann, 2004
Indriidae												
Propithecus diadema		154-160	1-2		175-210		0	0			30	Puschmann,2004
Propithecus tattersalli		154-160	1-2		175-210		0	0			30	Puschmann, 2004
Daubentoniidae												
Daubentonia madagascariensis		158-176	1		280-350	100-130	0	0				Puschmann, 2004
Lorisidae												

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Arctocebus calabarensis	403	133	1	1		25.3	0	0				Leutenegger, 1973
Loris tardigrades		163-174	1-2			10-12	0	0			10-12	Puschmann, 2004
Nycticebus coucang	1230	193	1	1	90-180	45	0	0			17-20	Leutenegger, 1973
Perodicticus potto	1348	193	1	1	119-175	48.5	0	0			9-18	Leutenegger, 1973
Galagidae												
Galago crassicaudatus	1030	133	2			46.4	0	0			12-20	Leutenegger, 1973
Galago demidovii	60	100-120	1		56	7.6	0	0			8-10	Leutenegger, 1973
Galago senegalensis	229	122-125	1		30	23.2	0	0			12	Gucwinska & Gucwinska, 1968
Tarsiidae												
Tarsius syrichta	120	180	1		140	26.2	0	0				Leutenegger, 1973
Simiae												
Cebidae												
Alouatta belzebul		180-194	1			600	0	0			3.5 4 y	Puschmann, 2004
Alouatta seniculus		180-194	1			600	0	0			3.5 4 y	Puschmann, 2004
Alouatta villosa	7670	180-194	1			440	0	0			3.5 4 y	Ardito, 1975
Aotus lemurinus		180	1		240		0	0				Burnie, 2001
Aotus nigriceps		150-153	1-2			70-100	0	0			2 3 y	Puschmann, 2004
Ateles chamek		226-232	1-2		540	340-480	0	0			4-5 5-6 y	Puschmann, 2004
Ateles geoffroyi	7600	225	1		330	512	0	0			4-5 5-6 y	NZP records

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀	♂	References
Brachyteles arachnoides		210-255	1				0	0					Puschmann, 2004
Brachyteles hypoxanthus		210-255	1				0	0					Puschmann, 2004
Cacajao roosevelti			1				0	0			3.5	5.5 y	Puschmann, 2004
Callicebus brunneus		150-155	1			70	0	0			2-3	2-3.5 y	Puschmann, 2004
Callicebus torquatus		150-155	1			70	0	0			2-3	2-3.5 y	Puschmann, 2004
Cebus apella		149-167	1-2			200	0	0			4-5	6-7 y	Puschmann, 2004
Cebus capucinus	2700	180	1	0.5		230	0	0			4-5	6-7 y	Leutenegger, 1973
Lagothrix cana		233	1		180		0	0					Burnie, 2001
Lagothrix flavicauda		225	1				0	0			4-5	5-6 y	Puschmann, 2004
Lagothrix lagotricha	5400	225	1	0.5	9-12 mo	450	0	0			8 y		Williams, 1967
Pithecia aequatorialis		163-176	1				0	0			2	3 y	Puschmann, 2004
Pithecia pithecia	1046	163	1				0	0			2	3 y	Leutenegger, 1973
Saimiri boliviensis		155-170	1-2			90-115	0	0			3	4 y	Puschmann, 2004
Saimiri sciureus	590	157	1			84	0	0			3	4 y	Leutenegger, 1973
Callitrichidae													
Callimico goeldii	472	154	1	1	70-84	40	0	0			12-18		Leutenegger, 1973
Callithrix argentata			2		56-84	26-28	0	0			13	13-16	Puschmann, 2004
Callithrix geoffroyi			2		56-84	26-30	0	0			13	13-16	Puschmann, 2004
Callithrix jacchus	241	144	2		180	27.5	0	0			13		Rothe, 1977
Callithrix penicillata			2		56-84		0	0			13	13-16	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Cebuella pygmaea	145	145	2		70-91	16	0	0			14-18	Leutenegger, 1973
Leontopithecus chrysomelas			2		84-98	53-68	0	0			12-18	Puschmann, 2004
Leontopithecus rosalia	745	128	2	1-2	90	61	0	0			12-18	NZP records
Saguinus fuscicollis		145-152	2		70-84		0	0			18-22	Puschmann, 2004
Saguinus imperator		140-145	2		70-84		0	0			18-22	Puschmann, 2004
Saguinus labiatus		140-150	2	1-2	70-84	51-56	0	0			18-22	Puschmann, 2004
Saguinus leucopus			2		70-84	47-61	0	0			18-22	Puschmann, 2004
Saguinus midas	483	140	2	1	70-84	42-50	0	0			18-22	Leutenegger, 1973
Saguinus oedipus	510	140	2	1	70-84	40	0	0			18-22	Leutenegger, 1973
Saguinus tamarin	483	140	2	1	70-84	34	0	0			18-22	Leutenegger, 1973
Cercopithecidae												
Cerocebus atys		173	1		6-10 mo		0	0			2-3 4-5 y	Puschmann, 2004
Cercopithecus aethiops		163-170	1	0.5-1	6 mo	300-400	0	0			2-2.5 3 y	Puschmann, 2004
Cercopithecus ascanius		165-170	1		6 mo	230	0	0			4-4.5 5-6 y	Puschmann, 2004
Cercopithecus campbelli		170-180	1			300	0	0			3-3.5 4-5 y	Puschmann, 2004
Cercopithecus diana		170-185	1			330-410	0	0			4 4-5 y	Puschmann, 2004
Cercopithecus hamlyni		169-183	1		6 mo	250-350	0	0			3.5-4 4-5 y	Puschmann, 2004
Cercopithecus mitis			1			400	0	0			4-5 5-5.5 y	Puschmann, 2004
Cercopithecus mona		170-180	1			300	0	0			3-3.5 4-5 y	Puschmann, 2004
Cercopithecus neglectus		168-187	1			300-550	0	0			3.5-4 5-6 y	Puschmann, 2004
Colobus guereza		198-202	1		9-12 mo	365-590	0	0			4 6-7 y	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Colobus polykomos		186-205	1		9-12 mo	400-530	0	0			2.5-3.5 y	Puschmann, 2004
Erythrocebus patas		160-170	1		12-17 mo	420-500	0	0			2.5-3 3-4 y	Puschmann, 2004
Lophocebus albigenus		174-180	1		6-10 mo		0	0			3-4 5-7 y	Puschmann, 2004
Macaca arctoides		178	1		9-10 mo	350-600	0	0			3.5-4 y	Puschmann, 2004
Macaca fascicularis		165	1		8-12 mo	230-470	0	0			3.5-4 y	Puschmann, 2004
Macaca fuscata		170-180	1		5-10 mo	450-550	0	0			3.5-4 y	Puschmann, 2004
Macaca mulatta	4370	167	1		7-10 mo	330-600	0	0			2-3 3-4 y	Ardito, 1975
Macaca nemestrina		170	1		8 mo	500	0	0			3.5-4 y	Puschmann, 2004
Macaca nigra		175	1			800	0	0			4 5 y	Puschmann, 2004
Macaca radiata		163	1		8 mo	330-370	0	0			3-4 4-5 y	Puschmann, 2004
Macaca silenus		177	1		8-10 mo	350-370	0	0			3-4 4-5 y	Puschmann, 2004
Macaca sylvana		164	1		4-6 mo	350-450	0	0			4-5 y	Puschmann, 2004
Mandrillus leucophaeus		174-183	1		8-15 mo		0	0			3-3.5 4-5 y	Puschmann, 2004
Mandrillus sphinx		172-183	1		7-10 mo	470	0	0			3-3.5 5-6 y	Puschmann, 2004
Miopithecus ogouensis		158-166	1	1	4-5 mo	150-175	0	0			4 5.5-6.5 y	Puschmann, 2004
Nasalis larvatus	9873	166	1	0.5-1	7 mo	450	0	0			4 y	Pournelle, 1967
Papio anubis		175	1	0.6	6-8 mo	450-1050	0	0			3.5-4 4-5 y	Puschmann, 2004
Papio hamadryas		173	1	0.6	6-8 mo	420-800	0	0			3-4 4.5-5 y	Puschmann, 2004
Papio papio		175	1	0.6	6-8 mo	450-1050	0	0			3.5-4 4-5 y	Puschmann, 2004
Papio ursinus		175	1	0.6	6-8 mo	450-1050	0	0			3.5-4 4-5 y	Puschmann, 2004
Piliocolobus gordonorum		198	1	0.5	10-12 mo		0	0			2.5-3 4-5 y	Puschmann, 2004
Presbytis obscurus	5400	150	1			485	0	0				Ardito, 1975
Pygathrix nemaeus		190-210	1	0.5	10-13 mo	400	0	0			4 5 y	Puschmann, 2004
Semnopithecus entellus		195-215	1		10-12 mo	500	0	0			2.5-3 6-7 y	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Theropithecus gelada	9830	170	1	0.5	9-12 mo	464	0	0			3-4 5-7 y	Ardito, 1973
Trachypithecus auratus		170	1		12-15 mo	290-410	0	0			3-4 4 y	Puschmann, 2004
Trachypithecus francoisi		195-215	1		11-12 mo	400-550	0	0			3-3.5 4-6 y	Puschmann, 2004
Trachypithecus obscurus		200-210	1	0.5	7-9 mo	400-500	0	0			2.5-3 y	Puschmann, 2004
Trachypithecus vetulus		195-210	1		8-10 mo	360	0	0				Puschmann, 2004
Hylobatidae												
Hylobates lar	6800	225	1		20 mo	400	0	0			5-7 8-9 y	NZP records
Nomascus concolor		199-216	1	0.5	20 mo	300-450	0	0			5-7 8-9 y	Puschmann, 2004
Nomascus nasutus		199-216	1	0.5	20 mo	300-450	0	0			5-7 8-9 y	Puschmann, 2004
Symphalangus syndactylus	10300	223	1		20 mo	560	0	0			5-7 8-9 y	NZP records
Pongidae												
Gorilla beringei		267	1		24 mo	2100	0	0			9-11 6-7 y	Puschmann, 2004
Gorilla gorilla	75000	252-255	1		14 mo	2040	0	0			9-11 6-7 y	NZP records
Homo sapiens	60000	270	1	0.28		3300	0	0				Eisenberg, 1983
Pan paniscus		245	1	0.3	24 mo	1600	0	0			7-9 7 y	Puschmann, 2004
Pan troglodytes	39000	235	1	0.3	24 mo	1580	0	0			10 7-10 y	NZP records
Pongo pygmaeus	37000	263	1	0.25	36 mo	1200	0	0			7-8 6-7 y	NZP records
Dermoptera												
Cynocephalus volans		60	1		180	35						Puschmann, 2004
Scandentia												
Tupaiidae												
Tupaia belangeri	200	45	2	7-8	33-37	10	21.5	4			3	Martin, 1967
Tupaia glis		42-45	1-4 (2)	max. 7	38	10-14					3	Puschmann, 2004
Tupaia tana		45-48	1-4 (2)	max. 7	38	10-14					3	Puschmann, 2004

	Reproduction					Development							
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀	♂	References
GLIRES													
Lagomorpha													
Leporidae													
Brachylagus idahoensis	350	26-28	4-8	3									Burnie, 2001
Bunolagus monticulares	1400-1900	35-36	1-2			40							Mills & Hes, 1999
Lepus americanus	1500	37	3-5	2.4	15	70-80	0	0			7-8	5-7	Newson, 1964
Lepus arcticus		50	7	1-3	14-21		0	0			7-8	5-7	Puschmann, 2004
Lepus californicus	2100	41-47	2.3	4.3	14	69	0	0					Lechleitner, 1959
Lepus capensis	1500-2500	42	1-3	2-3	21	100	0	0			7-8	5-7	Mills & Hes, 1999
Lepus europaeus	3750	42-44	1-6	3-4	14-21	123	0	0			7-8	5-7	Flux, 1967
Lepus insularis		42	1-6		14-21		0	0			7-8	5-7	Puschmann, 2004
Lepus timidus	3000	44-53	1-7	2	28	87	0	0			7-8	5-7	Flux, 1970
Lepus townsendi		42	1-8	2	14-21	170	0	0			7-8	5-7	Puschmann, 2004
Oryctolagus cuniculus	2000	30-37	4-15	5-7	20	38	10				< 12		Flux, 1967
Romerolagus diazi		38-40	1-4	4-5	23	50	5-6						Puschmann, 2004
Sylvilagus aquaticus	2200	39-40	2.8	2	14	35-40	4-7				12		Hunt, 1959
Sylvilagus auduboni		30	2-6	2-4							80 d		Puschmann, 2004
Sylvilagus bachmanni		28-30	1-7	max. 5							12		Puschmann, 2004
Sylvilagus floridanus		27-30	1-12	4-5	25	35-40	4-7				12		Puschmann, 2004
Sylvilagus nuttali		28-30	3-8	max. 5							12		Puschmann, 2004
Ochotonidae													
Ochotona alpina		30	2-6 (3)	2-3	28	7-9	7-10	2			> 12		Puschmann, 2004
Ochotona pallasi		25	8	2-3	28	7-9	7-10	2			>12		Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Ochotona princeps		30-32	3-5	2-3	28	7-9	7-10	2			>12	Puschmann, 2004
Ochotona pusilla		20-24	7-13	3-4	28	7-9	7-10	2			>12	Puschmann, 2004
Rodentia												
Sciurognathi												
Aplodontidae												
Aplodontia rufa	364	28-30	2-3		56	18-30	50				12	Puschmann, 2004
Sciuridae												
Citellus citellus		25-28	6	1	35-42	6	28				11	Mohr, 1954
Citellus columbianus	336	24	4.5	1	28	7-9	21-23				11	Shaw, 1925
Citellus tridecemlineatus	152	28	8	1.5	29	2.6-4	27				11	Johnson, 1931
Cynomys ludovicianus	1200	27-33	5	1	49	15	33-37				22	Koford, 1957
Eutamias amoenus	47	32	5.25	1		2.6	31.5					Broadbrooks, 1958
Eutamias quadrivittatus	115	31.5	5.25	1		2.8	31					Wadsworth, 1969
Eutamias sibiricus		28-31	4-6		42-49	4	26-29				10-11	Puschmann, 2004
Funambulus layardi		40-45	1-5 (3)		56						12	Puschmann, 2004
Glaucomys sabrinus	138	37	4	2	65	5.8	31				24	Muul, 1969
Glaucomys volans	60	39	3	1	60-70	3.6	26				12	Sollberger, 1943
Marmota marmota		33-34	2-7		49-56		20-26				22	Puschmann, 2004
Marmota monax	5500	31-32	4	1	56-70	25-30	26-28				24	Hamilton, 1934
Paraxerus alexandri		56-58	2-3		28-42						6-10	Puschmann, 2004
Paraxerus palliatus		56-58	2-3	1	28-42		7-10				6-10	Mills & Hes, 1999
Petinomys genibarbis		53	1				0					Puschmann, 2004
Pteromys volans		40	1-5		28-42		14					Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Sciurus caolinensis	425	44	2.7	2	49-70	16.5	37				124 d	Horwich, 1972
Sciurus niger	750-950	45	3	2	49	16	42				10-11	Asdell, 1964
Sciurus vulgares	300	38	3-7	2	43-70	11.5	31				10-11	Mohr, 1954
Spermophilus citellus		25-26	6-8		35-42	6	21				11	Puschmann, 2004
Tamias striatus	86	31	3-5	1-2	35	2.5	31				10-11	French et al., 1975
Tamiasciurus hudsonicus	190	40	3.3	1-2	50	7-8	26-34				13-15	Dolbeer, 1973
Xerus inauris	1000	48	2-6	1		20					6	Mills & Hes, 1999
Xerus princeps	1400	48	3	1								Mills & Hes, 1999
Castoridae												
Castor canadensis	24000	128	4	1	42	340	0	0			2-3 y	Bradt, 1938
Castor fiber		105-107	1-7 (3)		90	450	0	0			2 y	Puschmann, 2004
Geomyidae												
Geomys breviceps	200	28	2	1.7	60	5.8	26				6	Wood, 1949
Geomys bursarius		18-19	3-4		60	2.8-4	26				6	Puschmann, 2004
Thomomys bottae		29	5.8	1-2	60	4.1	26				12	Scheffer, 1938
Heteromyidae												
Dipodomys deserti		29-33	3-4		24-29	3-8						Puschmann, 2004
Dipodomys merriami	35	33	2		20		11-12					Eisenberg, 1963
Dipodomys nitratoides	37	32	2-3		23	4	10					Eisenberg, 1963
Dipodomys ordii		29-30	3-4		24-29	3-8					2	Puschmann, 2004
Dipodomys panamintinus	65	29	3-4		28	4.5	17-18					Eisenberg, 1963
Liomys pictus	44	25	4		26	2.5	19				3	Eisenberg, 1963
Liomys salvini		27-28	3-4		24-29	2.5-3						Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Perognathus californicus	22	25	3.5		23	1.5	15					Eisenberg, 1963
Perognathus parvus	23	24	5	1-2	24-29	1.5	15					Eisenberg, 1967
Dipodidae												
Jaculus orientalis	130-225	29	2	3-4			35					NZP records
Sicista betulina	8	31.5	2-6	1	34-36		26-28				12	Mohr, 1954
Muridae												
Acomys spinosissimus	33	35-37	2-5 (3)	max.10	19-20	6-6.5	0-2	4-8				Puschmann, 2004
Acomys subspinosus	22	35-37	2-5	max.10	19-20	6-6.5	0-2	4-8				Puschmann, 2004
Aethomys chrysophilus	101	21-23	2-7 (4)									Mills & Hes, 1999
Apodemus agrarius	20.5	22	2-8	3-4	14-15	2.5	12.5				49-56 d	Mohr, 1954
Apodemus flavicollis	27	24.5	3-8	2-3	20-22	2.5	14				49-56 d	Mohr, 1954
Apodemus sylvaticus	19.5	23	2-9 (6)	4-5	21	2.5	14				60 d	Mohr, 1954
Arvicola terrestris	120	21	2-7 (6)	3-4	12-16	10	9.5	14			63 42 d	Mohr, 1954
Baiomys taylori	7.9-9.4	18	2.7			1.1	12					Hudson, 1974
Cannomys badius	325	41.5	2			7	23.5					Eisenberg & Maliniak, 1973
Clethrionomys gapperi		17-21	4-6	3-4		1.9-2	12-13	12			56 42 d	Puschmann, 2004
Clethrionomys glareolus	23	21	4-7 (5)	3-4	18	1.6	12	12			56 42 d	Asdell, 1964
Cricetomys gambianus	1300	27	2-4	max.10								Mills & Hes, 1999
Cricetus cricetus	150-250	20	11	2-4	18	5-8	14	12			43 d	Mohr, 1954
Desmodillus auricularis	50-55	21	4		21							Mills & Hes, 1999
Dicrostonyx groenlandicus	76	19-21	3-4	max. 5	15-20	4	12-14				49 84 d	Puschmann, 2004
Gerbillurus paeba	25	21	4		15-30		14-18	8-10				Mills & Hes, 1999
Gerbillurus tytonis			4-5		23-30		20-24					Mills & Hes, 1999

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Gerbillus nanus	22	21-23	3-7	max. 3			13.5					Eisenberg, 1967
Gerbillus pyramidum	65	21	4	max. 3		2.6	18.5					Petter, 1961
Grammomys cometes			2-5 (3)	3	19	4	10					Mills & Hes, 1999
Lagurus lagurus		24-25	5-8	5		1.5						Puschmann, 2004
Lemmus sibiricus		18	7-8	3-9	14-16	3.3-4	11	12			21 49 d	Puschmann, 2004
Lemniscomys rosalia	80	21	5-7	max.14		3						Mills & Hes, 1999
Malacothrix typica	16				32	1					70 d	Mills & Hes, 1999
Mastomys coucha	56-70	23	10-16	12	21-23	1.8-2.2	15				100 d	Mills & Hes, 1999
Mastomys natalensis	56-70	23	10-16	12	21-23	1.8-2.2	15				100 d	Mills & Hes, 1999
Meriones crassus	105	21	3-6	3-4	20	2.5-5.5	17	15			28-35 d	Koffler, 1972
Meriones libycus	100	24	2-7	3-4	20	2.5-5.5	14.3	15			28-35 d	Petter, 1961
Meriones persicus	110	22-23	4	3-4	20	2.5-5.5	16-17	15			28-35 d	Eibl-Eibesfeldt, 1951
Meriones shawi	185	21	4	3-4	20	4.8	18	15			28-35 d	Petter, 1961
Meriones tristrami	105	24	3-4	3-4	20	2.5-5.5	15.5	15			28-35 d	Petter, 1961
Meriones unguiculatus	53	23	5.1	3-4	30	2.8	16-17	15			28-35 d	NZP records
Mesembriomys gouldi	1000	43-44	1-3			35	11					Crichton, 1969
Mesocricetus auratus	80-100	16	11	max. 8	21	1.8	12	10-11			56-70 d	NZP records
Micromys minutus	4-7	21	11	3-9	18-20		8-9	18			35-49 d	Mohr, 1954
Microtus agrestis	30	21	4-7	3-4	12-14	2-3	9.5	10			21 28 d	Mohr, 1954
Microtus arvalis	22	21	8	3-7	16	1.8	9.5	10			14 28 d	Mohr, 1954
Microtus nivalis	43	21	4-7	2	17-20	3.3-4.2	10	10			14 28 d	Mohr, 1954
Microtus oeconomus		20-22	2-7	max. 9	17-20	1-3.2	10	10			14 d	Puschmann, 2004
Microtus pennsylvanicus	35	21	4		14	2.6	7-11	10			14 28 d	Asdell, 1964

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Microtus subterraneus		21	1-5 (3)		15-17	2	11-14	10				Puschmann, 2004
Mus minutoides	7.8	19	1-7 (4)		17	1	12-14	8-10				Mills & Hes, 1999
Mus musculus	20.5	19	5	4-6	28	1.5	12	15			28 42 d	Mohr, 1954
Mystromys albicaudatus	99-145	38	3		38	6.5	16-20				32 d	Hall et al., 1967
Neotoma albigula	198	38	2.2	max. 5	30	10.9	15					Egoscue, 1957
Neotoma cinerea	271	27-32	3.5	max. 5	21	12-14	14-15					Martin, 1973
Neotoma fuscipes		33	2-3	max. 5		12-14	17					Linsdale & Tevis, 1951
Nyctomys sumichrasti	55	34	2			4.7	16.5					Birkenholz & Wirtz,1965
Ochrotomys nutalli	24.7	29-30	2.6		18	2.8	12.7					Linzey & Linzey, 1967
Ondatra zibethicus	1090	30	8	4-5	17	21	11				84-240 d	Asdell, 1964
Onychomys leucogaster	30	27-32	3.7	max.12	33-47	2.6	18				42 84 d	NZP records
Ototylomys phyllotis	63.8	50-52	2.3		53.6	10.2	4					Helm, 1973
Pachyuromys duprasi	50	21	4.5				20.5					Petter, 1961
Peromyscus californicus	38	23.6	1-3	3-4	21	4.3	15				35 63 d	Layne, 1968
Peromyscus crinitus	15.1	24	3.7	2	28	2.2	13.5				35 63 d	Layne, 1968
Peromyscus eremicus	22	21	1-4 (3)	max.14	35	2	13				35 63 d	Layne, 1968
Peromyscus gossypinus	29	23	3.7	max.14	20-30	2.2	10-18				30 d	Layne, 1968
Peromyscus leucops	22	23	5	max.14	20-30	1.87	13				23 d	Layne, 1968
Peromyscus maniculatus	15	25	3-4	2-4	18	1.1-2.3	13.7				35 63 d	Layne, 1968
Peromyscus megalops	71		1.6	max.14	21	3.9	22.8				35 63 d	Layne, 1968
Peromyscus polionotus	15	23-24	3.3	3.4	20-30	1.1-2.2	13.7				35 63 d	Layne, 1968
Peromyscus truei	27	26.2	3.4	max.14	20-30	2.34	14				40 d	Layne, 1968

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Peromyscus yucatanicus		27-31	3.5	max.14	20-30	2.5	13-21				35 63 d	Lackey, 1976
Petromyscus collinus	20		2-3	1								Mills & Hes, 1999
Pitmys subterraneus	17.5	21	2-5	5-6	15-17	2	12.5				2-3	Asdell, 1964
Phodopus roborovskii		19-21	2-10 (5)	max.12	18-21	1	14	12			1	Puschmann, 2004
Psammomys obesus	212	25					15.5					Petter, 1961
Rattus norvegicus	330	22	8	2-7	28	4.92	15	18			35 63 d	Mohr, 1954
Rattus rattus	187.5	22	6-8	2	21	4.5-6	14.5	14			3	Mohr, 1954
Reithrodontomys megalotis	10-15	23					7-8					Leraas, 1938
Reithrodontomys montanus	8.5	23-24	2-4			1.0-1.3	8					Hall & Kelson, 1959
Rhabdomys pumilio	55	25	2-9 (6)				7				56-63 d	Mills & Hes, 1999
Saccostomus campestris	48	20-21	5-10(7)		21	2.8						Mills & Hes, 1999
Scotinomys tegurina	15	30	3			2.2	12-15					Eisenberg, 1983
Scotinomys xerampelinus	15	32	2-4			2.2	17.8					Eisenberg, 1983
Sigmodon hispidus	215	27	5-6		20	7.2	0					Meyer & Meyer, 1944
Tachyoryctes ruandae	250	47.5	1-2			14	22					Rahm, 1969
Tatera indica	110	24	11	3-4			18					Eisenberg, 1967
Zelotomys woosnami	57		5-11				16-17					Mills & Hes, 1999
Pedetidae												
Pedetes cafer		80-82	1-2	3-4	45	230-320	4-6	0			12	Puschmann, 2004
Pedetes capensis	3000	77	1	3	49		4-6	0			8	Mills & Hes, 1999
Ctenodactylidae												
Ctenodactylus gundi		55	1-2	2		40	0	0			12	Puschmann, 1989

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Myoxidae												
Dryomys nitedula	92.5	24.5	3	1-2			21					Mohr, 1954
Eliomys quercinus	72.5	23	5	1-2	28-34		19.5					Mohr, 1954
Glis glis	92.5	30-32	5	1-2	25-35		20-22					Mohr, 1954
Muscardinus avellanarius	20	20	6	1-2	28-35		17				196-280 d	Mohr, 1954
Hystricognathi												
Bathygeridae												
Cryptomys hottentotus	60-140	60	4	1-2								Mills & Hes, 1999
Hystricidae												
Acanthion hodgsoni		105	1	2-3	60	260	0	0			24	Puschmann, 2004
Atherurus africanus	2500	105	1	2-3	60	150	0	0			24	Rahm, 1962
Hystrix africaeaustralis	12000-18000	93-105	1-4	2-3	60	300-400	0	0			24	Puschmann, 2004
Hystrix cristata	20000	112	1	2-3	60	150	0	0			24	NZP records
Hystrix galeata		93-105	1-4	2-3	60		0	0			24	Puschmann, 2004
Hystrix leucura		93-105	1-4	2-3	60	400-450	0	0			24	Puschmann, 2004
Petromuridae												
Petromus typicus	190		1-2				0	0				Mills & Hes, 1999
Thryonomyidae												
Thryonomys swinderianus	2000	155	4	2	30	130	0	0			12	Weir, 1974
Ctenomyidae												
Ctenomys peruanus		120	1-7	1	35	8	0	0			8	Puschmann, 2004
Ctenomys talarum	150	130	4	1	35	8	0	0			8	Weir, 1974
Ctenomys torquatus		107	1-7	1	35	8	0	0			8	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Octodontidae												
Octodon degus	250	99	5	2	28	14	0	0			6	NZP records
Octodontomys gliroides	100	99	2	2	35	15	0	0			3-4	NZP records
Abrocomidae												
Abrocoma cinerea	140	115-118	2			15-18	0	0				NZP records
Echimyidae												
Proechimys guairae	300	63	3		21	22	0	0			3 2	Weir, 1974
Proechimys guayannensis		62-64	1-7 (3)		21-35		0	0			146 d	Puschmann, 2004
Proechimys semispinosus	324-350	64.8	2.8		21	23	0	0			100 d	Maliniak & Eisenberg, 1971
Trichomys aperoides		89	1-7 (3)		40	30	0	0			2 3	Puschmann, 2004
Chinchillidae												
Chinchilla brevicauda		120-128	1-4 (2)	2	35-42	35	0	0			3-5	Puschmann, 2004
Chinchilla laniger	500	111	2	2	49	35	0	0			5-8	Weir, 1974
Lagidium boxi	1400	140	1	2-3	42-56	260	0	0			5.5	Weir, 1974
Lagidium peruanum	1200	140	1	2-3	56	180	0	0			5.5	NZP records
Lagostomos maximus	3000	153	2	2-3	30	200	0	0			5-6	Weir, 1974
Dinomyidae												
Dinomys branickii	13000	222-283	2			900	0	0				NZP records
Caviidae												
Cavia aperea	500	61	3	4-5	21	60	0	0			3 2	Weir, 1974
Cavia porcellus	1000	68	6	4-5	21	100	0	0			3 2	Weir, 1974
Dolichotis patagona	7856	93	1.5	3-4	70-77	432	0	0			5-6	Weir, 1974
Dolichotis salinicola		77	1	3-4	70-77		0	0			5-6	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Galea musteloides	400	52	3	4-7	21	40	0	0			3 2	Weir, 1974
Kerodon rupestris		77	1-2	2	21	80	0	0				Puschmann, 2004
Pediolagus salinicola	2000	77	1	3-4	70-77	193	0	0			5-6	NZP records
Microcavia australis		54	1-5	4-5	21	30	0	0			3	Puschmann, 2004
Capromyidae												
Capromys melanurus		140			150		0	0			10	Puschmann, 2004
Capromys pilorides	4300	120	2	1	150	220	0	0			10	NZP records
Erethizontidae												
Erethizon dorsatum	5000	217	1		56	1500	0	0			2-2.5 y	Weir, 1974
Hydrochaeridae												
Hydrochaeris hydrochaeris	30000	150	4	1	112	1500	0	0			15	Weir, 1974
Dasyproctidae												
Dasyprocta spec.	2700	120	1.5	2	20	200	0	0			9	Weir, 1974
Myoprocta pratti	1000	99	2	2	14-20	100	0	0			8-12	Kleiman, 1972
Agoutidae												
Agouti paca	8200	115	1.5			702	0	0				NZP records
Geocapromys ingrahami	700	100	1	1		80	0	0				Weir, 1974
Plagiodontia aedium	1970	135-150	1	1		100	0	0				NZP records
Myocastoridae												
Myocastor coypus	6000	132	5	2-3	56	225	0	0			3-4	Weir, 1974
LAURASIATHERIA												
Eulipothyphla												
Solenodontidae												

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Solenodon paradoxus	800	84	1-2	2	75	60		14				Eisenberg, 1975
Solenodon cubanus	600	84	1-2	2	75	40-55		14				Puschmann, 2004
Erinaceidae												
Atelerix frontalis	240-400	35-42	1-9 (4)		35							Mills &Hes, 1999
Atelerix pruneri		37-38	5-7	3							9-11	Puschmann, 2004
Erinaceus europaeus	800-1025	34-49	1-7	2	21-28	13.9	14	20			9-11	Herter, 1957
Erinaceus roumanicus		31-35	5-7	2							9-11	Puschmann, 2004
Paraechinus micropus		31-35	1-2	3	60		21				9-11	Herter, 1957
Soricidae												
Blarina brevicauda	17	20.3	5.4	3-4	20.6	0.8	12				1-2	Innes, 1994
Crocidura bicolor	5.7		4	3-4	19.3	0.8	10				2.5-3	Ansell,1964;
Crocidura canariensis	7.5	30	2.1			0.8						Innes, 1994
Crocidura flavescens	24	28-36	1-7 (4)		18-22	2						Mills &Hes ,1999
Crocidura fuscomurina	5		2-5		18-24		10-12					Mills &Hes ,1999
Crocidura hirta	15.1	22	3.9	3-4	18	1	13				2.5-3	Ansell,1964
Crocidura leucodon	7.9	31.5	3-7 (4)	3-4	20.5	0.8	11.7	3			2-3	Hellwing, 1973
Crocidura olivieria	34.9		3.1		23.5	2.1	15					Innes, 1994
Crocidura parva	5	22	4.2		20	0.3	14.3					Innes, 1994
Crocidura russula	11.2	29.7	1-7 (3.8)	3-4	20	1	10.7				2.5-3	Vogel, 1972; Innes, 1994
Crocidura suaveolens	7.7	27.6	4	3-4	18	0.7	10.8				2.5-3	Innes, 1994
Crocidura viaria	16.5		3.5		20	1.4						Innes, 1994
Cryptotis parva	2.8-5.6	21-23	4-6	3-4	21-22	0.3-0.4	14				2.5-3	Hamilton, 1944

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Myosorex varius	12.5		2.9		22	1	17					Innes, 1994
Neomys anomalus	14		7.2		28	0.6	22					Innes, 1994
Neomys fodiens	14.9	20.8	3-8 (6.3)	3-4	29.3	0.7	22				2.5-3	Price, 1953; Innes, 1994
Sorex araneus	9.9	21.5	5-7 (6.7)	1.5	23.4	0.5	19.3				280 d	Crowcroft, 1957; Innes, 1994
Sorex cincereus	4.4		6.7	3-4	20	0.3	18				2.5-3	Pruitt, 1954
Sorex coronatus	9.4	24	4.9		30	0.6						Innes, 1994
Sorex isodon	11.9	18	6.7		22	0.9	16					Innes, 1994
Sorex minutus	4.4	25	6.1	1.5	26.3	0.25					2.5-3	Hutterer, 1976
Sorex vagrans	6.6	20	5.2	3-4	22	0.4	21				2.5-3	Johnston & Rudd, 1957
Suncus etruscus	2.0	28	3.9	6	19.5	0.2	14.5				2.5-3	Fons, 1973
Suncus murinus	42.8	30.2	1.84	3-4	18.9	2-2.3	8.6	4			2.5-3	Dryden, 1968
Talpidae												
Parascalops brewerie		28-42	4	1	28		21				12	Eadie, 1939
Scalopus aquaticus		42	2-5	1			21				12	Arlton, 1936
Scapanus townsendi			2-4	1			21				12	Ingles, 1965
Talpa europaea	85-110	30-40	3.8	1			22				6	Godfrey & Crowcroft, 1960
Chiroptera												
Pteropodidae												
Cynopterus sphinx	90	115-125	1	1	60	12-14	0	0			12	Gopalakrishna, 1969
Eidolon helvum	280	120	1	1	60		0	0			12	Gopalakrishna, 1969

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Pteropus geddeiri	650	180	1	1	60		0	0			12	Gopalakrishna, 1969
Pteropus giganteus	700	145	1	1	60		0	0			12	Gopalakrishna, 1969
Pteropus niger		142	1	1	60		0	0			12	Gopalakrishna, 1969
Rousettus leschenaulti	70	125	1	1	60	12	0	0			12	Gopalakrishna, 1969
Rhinopomatidae												
Rhinopoma kinneari	31	123			60						12	Gopalakrishna, 1969
Megadermatidae												
Megaderma lyra	42.5	155	1	1	60	7-8	0				12	Gopalakrishna, 1969
Rhinolophidae												
Hipposideros bicolor	8.5	42.5	1		60	1.2					12	Gopalakrishna, 1969
Phyllostomidae												
Atribeus jamaicensis			1	2	60		0				200 d	NZP records
Carollia perspicilliata	17.6	105-115	1	2	42	5	0				200 d	Eisenberg, 1983
Vespertilionidae												
Antrozous pallidus	26	63		1	33	3.1	9				12	Davis, 1969
Eptesicus fuscus	17		1	1	> 30	3.5					12	Kleiman, 1969
Eptesicus serotinus	28.3			1	60	5.8	7.8				12	Kleiman, 1969
Myotis lucifugus	9	55		1	60	2	0				12	Wimsatt, 1960
Myotis myotis	34	53		1	60						12	Eisenberg, 1983

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Nyctalus noctula	26	71.5	2	1	60	5.7	6				12	Kleiman, 1969
Pipistrellus ceylonicus	7.5	52.5	2		60	1.2-1.4	0				12	Gopalakrishna, 1969
Pipistrellus pipistrellus	6.5	44	2	1	60	1.4	4.8				12	Kleiman, 1969
Plecotus townsendii	8	56		1	60	2.1-2.7					12	Pearson et al., 1952
Scotophilus wroughtoni	19	110	2		60						12	Gopalakrishna, 1969
Tylonycteris robustula	8	87.5			60	1.25					12	Medway, 1972
Molossidae												
Tadarida brasiliensis	12	80.5		1	60						270 d	Asdell, 1964
Tadarida pumila	11.5		1	3	21							Mills & Hes, 1999
Pholidota												
Manidae												
Manis crassicaudata		65-70	1		90	240	0				24	Puschmann, 2004
Manis gigantea	25000-33000	65-70	1		90	400-500	0				24	Puschmann,2004 MacDonald, 2002
Manis temminckii		65-70	1		90	300-400	0				24	MacDonald, 2002
Manis tetradactyla	1200-2000	65-70	1		90	100-150	0				24	Puschmann, 2004
Manis tricuspis		139	1		90	140	0				24	NZP records
Carnivora												
Fissipedia												
Mustelidae												
Amblonyx cinerea		60-63	1-7 (4)	2-3	120-150	50-60	35				12-18	Puschmann, 2004
Arctonyx collaris		210-240	1-7(2-3)		84-98	60	41-48				18-24	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Eira barbara		63-67	1-4	1	70-84	75-95	35-47	21-28			18-24	Puschmann, 2004
Enhydra lutris			1	2-3		1400-2300	0				3.5-4 y	Puschmann, 2004
Gulo gulo		210-320	2-3		70	80-100	28-35				3-4 y	Puschmann, 2004
Ictonyx striatus		35-44	1-3	1	56	15					9	Puschmann, 2004
Lutra canadensis		56*	2.3	0.5	112-140	150	16-19	21-28			2.5-3 y	Ewer, 1973
Lutra lutra	7400	61-63*	2	2-3	180-210	70-109	35				24 18	Ewer, 1973
Lutrogale perspicillata		61-63	3-4	2-3	105-135		21-28				2.5-3 y	Puschmann, 2004
Martes flavigula		132-152	1-5			50-65	25-40				36	Puschmann, 2004
Martes foina		230-250	3-5	1	56	30	30-35				27-28	Puschmann, 2004
Martes martes	940	30*	3	1	42-46	30	32-36				22-24	Ewer, 1973
Martes zibellina		250-300	2-5	1	56	25-30	30-38				27	Puschmann, 2004
Meles meles	5060	42	1-4		84	84	10					Neal, 1958
Mellivora capensis		180	1-2	1	119	150	40-45				18-24	Puschmann, 2004
Mephitis mephitis		63	6-10	1	42-49	14	13-20	10-20			10-11	Ewer, 1973
Mustela erminea	45-77	28*	2	1	56-70	2.5-4	35	21-28			10	Ewer, 1973
Mustela eversmanni		38-42	4-6	1	56-63	4-6	28-30	21-28			9	Puschmann, 2004
Mustela frenata	102	23-24*	6-7	1		31	35-37	21-28				Ewer, 1973
Mustela lutreola		35-42	3-4	1	56-70	6-12		21-28			9	Puschmann, 2004
Mustela nirvalis	59	35-36*	4.8	2	35	2.3	26-30	21-28			4-5	Ewer, 1973
Mustela putorius	800	41-42*	6-7	1	42-56	10	28	21-28			9	Ewer, 1973
Mustela sibirica		28-42	4-5	1	56		30-35				10	Puschmann, 2004
Mustela vision	565	28-30*	4-6	1	56	6-12	35				12	Ewer, 1973
Pteronura brasiliensis		65-70	2-4	2-3	210	200-250	30				24	Puschmann, 2004
	* delayed implantation corrected for; actual time from conception to birth is much longer											

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Spilogale putorius		21	3-5	1	56	9.4	27-28	10-20			10-11	Ewer, 1973
Taxidea taxus	6000	42	2-3	1	42	116	40-45				18-24	Ewer, 1973
Vormela peregusna		56-63	4-8	1	56-63		28-30	21-28			9	Puschmann, 2004
Procyonidae												
Bassaricyon gabbii	2800	73-74	1			51	7	0				Ewer, 1973
Bassariscus astutus	870-1100	60	3		120	25	31-34	0			10-22	Richardson, 1942
Bassariscus sumichrasti		63-66	1-2 (1)		112-126	25-30	31-34	0			10-22	Puschmann, 2004
Nasua narica		67-79	3-4		70-84	80-180	11-15	0			10	Puschmann, 2004
Nasua nasua	5000	77	1-5		70-84	180	5	0			10	Ewer, 1973
Potos flavus	1970	112-120	1		82	117	15	0			18 30	Poglayen-Neuwall, 1962
Procyon cancrivorus		63-64	3-4		112-126	70	21-24	0			12-24	Puschmann, 2004
Procyon lotor	5270	63	3-7 (4)	1	70	62-98	18-24	0			12-24	Ewer, 1973
Ailuridae												
Ailurus fulgens	3000-4500	130	2	1	150	195	10	0			19	Roberts & Kessler, 1979
Ailuropodidae												
Ailuropoda melanoleuca	182000	140	1-2	1	150-180	104	46	6			6.5 6-7 y	Eisenberg, 1983
Ursidae												
Helarctos malayanus	80000	95-96	1-2	0.5	360-480	225	14	3-5			3-5 y	Puschmann, 2004
Melursus ursinus		202-225	1-2	0.5	360-480	480	29-35	3-5			2.5-3.5 y	Puschmann, 2004
Tremarctos ornatus		150-153	1-2		365-480	330	22-40	3-5			39	Puschmann, 2004
Ursus americanus	77270	67*	2.5	0.5	365-480	252-336	21	3-5				Ewer, 1973
	* delayed implantation corrected for; actual time from conception to birth is much longer											

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Ursus arctos	105000	70*	2.5	0.5	365-480	600	28	3-5			2.5 y	Ewer, 1973
Ursus maritimus	170000	231-247	2.5	0.5	720	765	33-39	3-5			2.5-4 y	Ewer, 1973
Ursus thibetanus		202-240	1-4 (2)	0.5	365-480	350	24-34	3-5			2.5 y	Puschmann, 2004
Viverridae												
Arctictis binturong		90-92	2-3	1	172	284-341	4	0			944 d	NZP records
Arctogalidia trivigata		45	1-4 (2-3)	max. 2	60		11-13				12	Puschmann, 2004
Chrotogale owstoni		75-87	1		90-150	80-135	4-15				24	Puschmann, 2004
Civettictis civetta		72-82	1-4	2	140	680	1-2				24	Mallinson, 1973
Cryptoprocta ferox	7000-12000	90	2-4	max. 2	112	100	12-15	0			3 2 y	Albignac, 1973
Eupleres goudotii	1600		1		60	150	0					Albignac, 1973
Fossa fossa	1430	86	1		70	65-70	0				24	Albignac, 1973
Genetta abyssinica		56-77	2-3	max. 2	60	77-100	8-12				24	Puschmann, 2004
Genetta tigrina	1673	60-70	1-3	1	56	71	12-18					Wemmer, 1977
Paradoxurus hermaphroditus		64-91	2-3	max. 2		100	10				9 12	Puschmann, 2004
Viverra zibetha		60-81	2-3	max. 2	60		8-10				12	Puschmann, 2004
Herpestidae												
Atilax paludinosus			3	2	45-60	200	9-14					Puschmann, 2004
Galidia elegans	655	85	1	2	56	50	6-8	0			24	Albignac, 1973
Helogale parvula		50-54	2-6	max. 6	75	20	8-12				12	Ewer, 1973
Herpestes auropunctatus		70-75	2-4	2	28-35	25-40	10-12				12	Larkin & Roberts, 1979
	* delayed implantation corrected for; actual time from conception to birth is much longer											

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Herpestes edwardsi	1320	56-63	2-4	2	75		16-17				12	Ewer, 1973
Herpestes ichneumon		56-63	2-4	2	75		8-12				12	Puschmann, 2004
Mungos mungo		60-62	3-5	2	75	60-96	8-12				12	Ewer, 1973
Mungotictis decemlineata	725	90-105	1	2	15-20	50	0				24	Albignac, 1973
Suricata suricatta		77	2-5	2	58	41.5	10-12				24	NZP records
Protelidae												
Proteles cristatus		89-92	3-4	1	60-90	120-200		0			18-24	Puschmann, 2004
Hyaenidae												
Crocuta crocuta	55300	110	1-2		525	1460	0	0			2-3 y	Pournelle, 1965
Hyaena brunnea	56800	90	1-3		540	680-805	8	0			1.5-2.5 y	Eisenberg, 1983
Hyaena hyaena		90-94	2-3		540	500-750	7-9	0			1.5-2.5 y	Puschmann, 2004
Canidae												
Alopex corsac		49-51	3-6		70	50-80	12-14	0			34	Puschmann, 2004
Alopex lagopus	6000	52	4-10	1	56-70	75	14-16	0			10-11	Ewer, 1973
Canis adustus		57-63	3-4	1	42-56	80-200	5-8	0			10-11	Puschmann, 2004
Canis aureus		60-63	2-6	1	35	80-200	5	0			10-11	Ewer, 1973
Canis latrans	11000	58-61	4	1	35	274	14	0			21-22	Bekoff & Jamieson, 1975
Canis lupus	28000	63	6.5	1	42-56	400	12-15	0			21-22	Ewer, 1973
Canis mesomelas		57-62	3-5	1	56-70	80-200	5-8				10-11	Puschmann, 2004
Cerdocyon thous		53	4.5	1.8	90	590	14				740 d	NZP records
Chrysocyon brachyurus	23000	60-65	1-3		105	431	12	0				Brady & Ditton, 1979
Cuon alpinus	17000	60-70	2-5			270	13-15	0			12	Ewer, 1973
Fennecus zerda	1500	50-52	3	1	56-70	28	8	0			9-10	Ewer, 1973

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Lycaon pictus	20000	72-73	7	2	56-84	365	14	0			12	Ewer, 1973
Otocyon megalotis	3200	60-75	2-5			123	6-8	0			10	Ewer, 1973
Nyctereutes procyonoides	7500	59-79	2-8		56	75	9-10	0			10-11	Ewer, 1973
Speothos venaticus	7000	65	6		90-120	157	14-17	0			10	Ewer, 1973
Urocyon cinereoargenteus		63	2-7	1		100		0				Ewer, 1973
Vulpes velox		49-52	4-5		70	50-80	12-14	0				Puschmann, 2004
Vulpes vulpes	9000	51-52	5-6	1	56-70	105	13-15	0			10-11	Ewer, 1973
Felidae												
Acinonyx jubatus	43000	92	3-4		120-180	270	7	0			2-2.5 y	Hemmer, 1976
Caracal caracal	7400	71	1-3	1-2	98-133	200	7	0			12-18 18	Hemmer, 1976
Felis chaus		67	3-4	1-2	84-112	110-210	14	0			20-22 22	Puschmann, 2004
Felis geoffroyi		73	2	2			14	0				NZP records
Felis margarita	2194	61	2-4	1-2		39	14	0				Hemmer, 1976
Felis nigripes	1620	67	1-2	1	56-70	60	7	0			21	Hemmer, 1976
Felis sylvestris	5165	68	1-8	1-2	42-49	100	11	0			10 22	Hemmer, 1976
Herpailurus yagouaroundi		70-75	1-3	1-2			14	0			2-2.5 2-3	Puschmann, 2004
Leopardus pardalis	12000	75	1-2	1-2	70-84	250	10	0			1.5-2 2-3 y	Hemmer, 1976
Leopardus wiedi		76-81	1-2	1-2		170	14	0			10	Puschmann, 2004
Leptailurus serval	9040	73	2-4	1-2	112-140	250	9	0			22	Johnstone, 1977
Lynx canadensis	13640	61	1-4	1-2	91-126	250		0			10-21 20	Hemmer, 1976
Lynx lynx		63	1-6	1-2	91-126	300-430	6	0			21-22 24	Hemmer, 1976
Lynx rufus		65	2-3	1-2	77-105	120-370	14	0			18-22 24	Puschmann, 2004
Neofelis nebulosa	20000	88	1-4	1-2	140	470	11	0			18-24 20	Hemmer, 1976
Octocolobus manul		67	4-6	1-2	63-77	70-100	14-21	0			24	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Panthera leo	151000	110	1-5	0.5	231	1400	6	0			1.75-2 2 y	Hemmer, 1976
Panthera onca	62000	101	1-4	0.5	154	800	8	0			2-3 3-4 y	Hemmer, 1976
Panthera pardus	39000	96	2-3	0.5	84	300	7	0			3 2.5 y	Hemmer, 1976
Panthera tigris	134000	103	1-4	0.5	90	1359	11	0			4-5 y	Hemmer, 1976
Prionailurus bengalensis	3270	66	1-4	1-2	63-84	80	10	0			18-24	Hemmer, 1976
Prionailurus viverrinus		63	2-3	1-2	91-119	170		0				Hemmer, 1976
Profelis aurata		75	1	1-2	91-105	185-235	14	0			10-22 18	Puschmann, 2004
Profelis temmincki		80	1	1-2		250	14	0			18-24 24	Puschmann, 2004
Puma concolor	43700	93	1-5	1	120-150	400	10	0			2-3.5 y	Hemmer, 1976
Uncia uncia	39000	99	1-4	0.5	56-70	470	8	0			2.5-3.5 y	Hemmer, 1976
Pinnipedia												
Otariidae												
Arctocephalus australis		330-365	1	1	365	5000	0	0			3-4 4-5 y	Puschmann, 2004
Arctocephalus pusillus	120000	388	1	1	245	7000	0	0			3-4 4-5 y	Bryden, 1972
Arctocephalus tropicalis	50000		1	1	98	4000	0	0			3-4 4-5 y	Bryden, 1972
Callorhinus ursinus	55000	273	1	1	84	5000	0	0			3 y	Bryden, 1972
Eumetopias jubatus	350000	270	1	1	84	20000	0	0			3-4 4-5 y	Bryden, 1972
Neophoca cinerea		330-365	1	1	365	6000-7000	0	0			3-4 4-5 y	Puschmann, 2004
Otaria byronia		330-365	1	1	365	11000-14000	0	0			3-4 4-5 y	Puschmann, 2004
Phocarctos hookeri		330-365	1	1	365		0	0			3-4 4-5 y	Puschmann, 2004
Zalophus californianus		342-365	1	1	365	5200-7500	0	0			3-4 4-5 y	Puschmann, 2004
Odobenidae												
Odobenus rosmarus	560000	330	1		700?	55000	0	0			4-5 7 y	Bryden, 1972

	Reproduction					Development							
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀	♂	References
Phocidae													
Cystophora cristata	270000	330-345	1	1	14-21	12000	0	0			4 y		Bryden, 1972
Erignathus barbatus	340000	323	1	1	21	45000	0	0			6	7 y	Bryden, 1972
Halichoerus grypus		255	1	1	14-21	14000	0	0			4-5	6 y	Bryden, 1972
Histriophoca fasciata		330-365	1	1	28	10000	0	0			3-4	4-5 y	Puschmann, 2004
Hydrurga leptonyx		240-270	1	1	60						2.5	4 y	Puschmann, 2004
Leptonychotes weddelli	370000	270	1	1	42	30000	0	0			3-4	4-5 y	Bryden, 1972
Lobodon carcinophagus	220000	255	1	1	35	25000	0	0			3-4	4-5 y	Bryden, 1972
Mirounga angustirostris		330-365	1	1	21-23	30000	0	0			3-4	4-5 y	Puschmann, 2004
Mirounga leonina	420000	240	1	1	24	40000	0	0			2	4 y	Bryden, 1972
Monachus monachus		330-365	1	1	21	20000	0	0			3-4	4-5 y	Puschmann, 2004
Monachus schauinslandi		330	1	1	35	16000	0	0			3-4	4-5 y	Bryden, 1972
Ommatophoca rossi		330-365	1	1	21		0	0			3-4	4-5 y	Puschmann, 2004
Pagophilus groenlandicus	140000	240	1	1	10-11	11000	0	0			5-8	8 y	Bryden, 1972
Phoca vitulina	65000	240	1	1	35-42	10000	0	0			3-4	4-5 y	Bryden, 1972
Pusa caspica		330-365	1	1	28	5000	0	0			3-4	4-5 y	Puschmann, 2004
Pusa hispida	65000		1	1	56-70	4000	0	0			3-4	4-5 y	Bryden, 1972
Pusa sibirica		330-365	1	1	28	3000	0	0			3-4	4-5 y	Puschmann, 2004
Perissodactyla													
Equidae													
Equus africanus		330-345	1		180-240	25000	0	0			2.5-3 y		Puschmann, 2004
Equus asinus	187973	340	1	1	180-240		0	0			2.5-3 y		Asdell, 1964
Equus burchelli	219000	330-360	1	1	180-240	32000	0	0			1.5-3 y		Wackernagel, 1966

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Equus grevyi		420	1	1	180-240	30000-40000	0	0			3.5-5 y	Puschmann, 2004
Equus hemionus		330	1	1	180-240	25000-30000	0	0			2.5-3 y	Asdell, 1964
Equus przewalskii		330-345	1	1	180-240	35000-45000	0	0			2.5-3 y	Puschmann, 2004
Equus zebra	166500	360	1	1	180-240	30000	0	0			2.5-3 y	Asdell, 1964
Tapiridae												
Tapirus bairdi	185450	390-420	1	0.5	300	9400	0	0			3 y	Eisenberg, 1983
Tapirus indicus	380000	394	1	0.5	300	7100	0	0			4 y	Richter, 1966
Tapirus pinchaque		393	1	0.6	300	4000-5400	0	0			3 y	Puschmann, 2004
Tapirus terrestris	175000	397	1	0.5	300	4500	0	0			3 y	NZP records
Rhinocerotidae												
Ceratotherium simum		510-540	1		420-480	35000-40000	0	0			4-5 y	Puschmann, 2004
Diceros bicornis		475	1	0.4	420-480	27500	0	0			5-6 y	NZP records
Rhinoceros unicornis		488	1		420-480	60000-65000	0	0			6 7-9 y	NZP records
Cetartiodactyla												
(Cetacea)												
Mystacoceti												
Balaenidae												
Balaena mysticetus	60,000,000	280	1	0.5	360		0	-			6 y	Bryden, 1972
Eschrichtiidae												
Eschrichtius robustus		360	1	0.5	210		0	-			8 y	Bryden, 1972

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Balaenopteridae												
Balaenoptera acutorostrata	5,854, 540	300	1	0.5	135		0	-			6 y	Bryden, 1972; Wandrey, 1992
Balaenoptera borealis	12,201, 286	330	1	0.5	150		0	-			7 y	Bryden, 1972 ; Wandrey, 1992
Balaenoptera musculus	93,869, 000	360	1	0.5	210	8,000,000	0	-			3.5-5 y	Bryden, 1972; Wandrey, 1992
Balaenoptera physalis	50,172, 680	370	1	0.5	210		0	-			3.5-5 y	Bryden, 1972 ; Wandrey, 1992
Megaptera novaeangliae	31,837, 850	345	1	0.5	315		0	-			6 y	Bryden, 1972 ; Wandrey, 1992
Odontoceti												
Iniidae												
Inia geoffrensis	85000-130000	255	1			6000-7000	0	-			3-4 y	Puschmann, 2004
Ziphiidae												
Hyperoodon ampulatus	10,000, 000	360	1	0.5	365		0	-			9 7-9 y	Bryden, 1972
Physeteridae												
Physeter catodon	13,896, 350	480	1	0.5	390		0	-			7-12 9-11 y	Bryden, 1972 ; Wandrey, 1992
Monodontidae												
Delphinapterus leucas	1,000, 000	420-450	1		180		0	-			5 8 y	Puschmann, 2004
Phocoenidae												
Phocaena phocaena	55500	315	1	0.5	365	5000-6000	0	-			3-4 y	Bryden, 1972 ; Wandrey, 1992

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Globicephalidae												
Globicephala melaena	1,800, 000	435	1	0.5	390-488		0	-			8-10 y	Bryden, 1972
Orcinus orca	2,500, 000	510	1	0.125	365		0	-			8-10 y	Wandrey, 1992
Delphinidae												
Delphinus delphis	75000	276	1	0.5	120	6500-7500	0	-			5 y	Bryden, 1972 ; Wandrey, 1992
Tursiops truncatus	155000	360	1	0.5	330-480	20000	0	-			6 y	Bryden, 1972
(Artiodactyla)												
Nonruminantia												
Suidae												
Phacochoerus aethiopicus	65000	171-175	1-4	1	126-147	860-910	0	0			13-14	Mohr, 1970
Potamochoerus larvatus		118-122	1-5	1		650-900	0	0				Puschmann, 2004
Potamochoerus porcus	72270	120	1-4	1		650-900	0	0				Mohr, 1970
Sus barbatus		120	4-8	1	98-112	630-750	0	0			7-9 5-8	Puschmann, 2004
Sus salvanius		100-110	2-6	2	98-112	133-250	0	0			20-24	Puschmann, 2004
Sus scrofa	75000	120	3-5	1	98-112	750-1200	0	0			18	Mohr, 1970
Tayassuidae												
Tayassu albirostris		156-162	1-3 (2)	1			0	0			24	Puschmann, 2004
Tayassu tajacu	13640	112-116	2	2	42-56		0	0			12	Knipe, 1957
Hippopotamidae												
Choeropsis liberiensis	272000	194	1	0.9	90	4900	0	0			3-5.5 y	Dittrich, 1976
Hippopotamus amphibius	1,277, 000	237	1	1	180	55000	0	0			3-4 y	Dittrich, 1976

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Tylopoda												
Camelidae												
Camelus batrianus	450000	406	1	0.5	300-330	44000	0	0			3 y	NZP records
Camelus dromedarius	570000	389	1	0.5	360	33000	0	0			3 y	Gaulthier-Pilters, 1974
Lama guanicoe		334-368	1-2	0.5-1	120-180	11000	0	0			11-15	Puschmann, 2004
Vicugna vicugna	50000	330	1	0.5	300	6350	0	0			386 d	Schmidt, 1973
Ruminantia												
Tragulidae												
Moschiola memina		140	1-2	1-2	90		0	0			5	Puschmann, 2004
Tragulus javanicus	1500	120	1	1-2	90	370	0	0			4-5	Davis, 1965
Tragulus napu	5000	162	1	1-2	21 ?	371-379	0	0			5	Romer, 1974
Cervidae												
Alces alces		240-250	1-3	1	150	11000-16000	0	0			2-3 y	Asdell, 1964
Axis axis	46000	210-225	1	1-2	120-150	1800-4000	0	0			18	NZP records
Axis porcinus		220-235	1	2	120-150		0	0			18	Puschmann, 2004
Capreolus capreolus		145	1-2	1	150-180	1400-1500	0	0			18	Puschmann, 2004
Cervus albirostris		230-250	1	1		8000	0	0			2.5 y	Puschmann, 2004
Cervus canadensis	200000	249-262	1	1	90-120	4000-9500	0	0			18	NZP records
Cervus elaphus		235	1	1	90-120	4000-9500	0	0			18	Puschmann, 2004
Cervus eldi		240	1	1	90	3500-5500	0	0			12	Puschmann, 2004
Cervus nippon		221	1	1	90-120	3000-3300	0	0			18	Puschmann, 2004
Cervus unicolor	162000	240	1	1-2	90-120	14300	0	0			18	NZP records

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Dama dama	39000	240	1	1	107	4500	0	0			16	Chapman & Chapman, 1975
Elaphodus cephalophus		210	1	1	90-120	1600	0	0				Puschmann, 2004
Elaphurus davidanus		250	1	1	120-150	7000	0	0			18	NZP records
Hydropotis inermis		170-190	1-2	1	60-90	600-800	0	0			6-12	Puschmann, 2004
Mazama americana	20000	220	1	1	75	567 ?	0	0			6-18	NZP records
Mazama gouazoubira	17000	206	1	1	75	1300	0	0			6-18	Thomas, 1975
Mazama rufina		217	1	1	75		0	0			7	Puschmann, 2004
Moschus moschiferus	10000	160	1-2	1-2	90	490-900	0	0			12	Frädrich, 1966
Muntiacus muntjak	18000	200-220	1	1.5	120	1240	0	0			18	Barrette, 1975
Muntiacus reevesi		200-220	1	2	120	500-650	0	0			18	Puschmann, 2004
Odocoileus hemionus	57000	203	1-2	1	90	3000	0	0			18	Linsdale & Tomich, 1953
Odocoileus virginianus	47730	204	1-2	1	150 ?	2000-4000	0	0			18	Taylor, 1956
Ozotoceros bezoarcticus		210-230	1	1	120	1300-2500	0	0			12	Puschmann, 2004
Pudu mephistophiles	5900		1	1	75-90	400	0	0			9-12	Hick, 1969
Pudu pudu	6000	210	1	1	< 65	500	0	0			9-12	Hick, 1969
Rangifer tarandus	105000	210-240	1-2	1	60	5897-6623	0	0			1-1.5 y	Lent, 1966
Giraffidae												
Giraffa camelopardalis	1,017, 000	420-450	1	0.5	270	47000-70000	0	0			3-4 y	Dagg & Foster, 1976
Okapia johnstoni		453	1-2	0.5	270	22500	0	0			2-3 y	Puschmann, 2004
Antilocapridae												
Antilocapra americana	40000	230-240	1-2	1		1800-2500	0	0			16 24	Müller-Schwarze & Müller-Schwarze, 1973

	Reproduction					Development							
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀	♂	References
Bovidae													
Addax nasomaculatus		252-270	1	1	240	4500-7600	0	0			13	24-25	Puschmann, 2004
Aepyceros melampus	42000	204	1	1	180	3970-5500	0	0			15	12	Dittrich, 1969
Alcelaphus buselaphus		235	1-2	1	180	4800-13000	0	0			18		Puschmann, 2004
Ammotragus lervia	66000	154-161	1	1-2	120	3730	0	0			18		Asdell, 1964
Antidorcas marsupialis		167-175	1-2	1-2	180	4000-5000	0	0			6-7	12	Puschmann, 2004
Antilope cervicapra		170-180	1-2	1-2	180	3500-5000	0	0			8-14	12	Puschmann, 2004
Bison bison		274	1	1	180	25000-30000	0	0			2-4 y		Puschmann, 2004
Bison bonasus		276	1	1	180	25000-30000	0	0			2-4 y		Puschmann, 2004
Bos gaurus	702730	270	1-2	1	180		0	0			25	30	NZP records
Bos grunniens	250000	258	1	<1		18000	0	0					NZP records
Bos javanicus		285-300	1	1	180	30000	0	0			2 y		Puschmann, 2004
Bos mutus		255-270	1	1	180	20000	0	0			1.5-2.5 y		Puschmann, 2004
Boselaphus tragocamelus	122730	247	1	1-2	180	3000-7000	0	0			12-18		NZP records
Bubalus arnee		300-340	1	1	180	20000	0	0			1.5-2.5 y		Puschmann, 2004
Bubalus bubalis	425000	307	1	1	180	3000	0	0			2-3 y		NZP records
Cephalophus dorsalis		228-244	1-2	1	120-150	1600	0	0			11-14		Puschmann, 2004
Cephalophus monticola		120	1-2	1	60	800	0	0			6	9	Puschmann, 2004
Cephalophus niger		126	1-2	2	120-150		0	0			11-14		Puschmann, 2004
Cephalophus rufilatus		223-245	1-2	1	120-150	760-1140	0	0			8.5-9	17	Puschmann, 2004
Cephalophus zebra		221-229	1-2	1	120-150	1600-1800	0	0			11-14		Puschmann, 2004
Cephalophus sylvicultor		260	1	1	28-42	6500	0	0			11-14		NZP records

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Connochaetes gnou		252	1-2	1	240	9900-13800	0	0			22 24	Puschmann, 2004
Connochaetes taurinus	219000	252	1	1	180-240	17000	0	0			24	Asdell, 1964
Damaliscus dorcas		223-236	1-2	1	180	4200-9100	0	0			24	Puschmann, 2004
Damaliscus lunatus		229-231	1-2	1	180	8350	0	0			24	Puschmann, 2004
Capra aegagrus		150-160	1	1-2	120	2000-3500	0	0			2.5 1.5 y	Puschmann, 2004
Capra falconeri		153-155	1-2	1	120	1500-2500	0	0			2.5 1.5 y	Puschmann, 2004
Capra ibex	52500	150-180	1	1	90	3500-5500	0	0			1.5-2.5 y	Asdell, 1964
Capricornis sumatraensis	120000	240	1-2	1	150-180	1300-4500	0	0			18	Asdell, 1964
Gazella dama		190-210	1	1-2	180	3100-5400	0	0			12	Puschmann, 2004
Gazella dorcas		173	1	1	180	1015-1690	0	0			21-22	Dittrich, 1969
Gazella gazella		160	1-2	1-2	180	2000-3000	0	0			7-8 24	Puschmann, 2004
Gazella granti	41300	199	1	1	180	5050-7310	0	0			18 21	Dittrich, 1969
Gazella leptoceros		169	1-2	1-2	180	1000-1800	0	0				Puschmann, 2004
Gazella soemmeringi		180-210	1	1-2	180	3500-4000	0	0			18-21	Puschmann, 2004
Gazella spekei		179	1-2	1-2	180	1000-1700	0	0			8-17	Puschmann, 2004
Gazella thomsoni	17500	145?	1	1	180	2700-3000	0	0			12 18	Asdell, 1964
Hemitragus jemlahicus		179	1-2	1	120		0	0			18	Puschmann,2004
Hippotragus equinus		259-280	1	1	240	13500-20000	0	0			24	Puschmann, 2004
Hippotragus niger	218640	271	1	1	240	11000	0	0			19.6	Dittrich, 1969
Kobus defassa	181000	280	1	1	180-240	12000	0	0				Dittrich, 1969
Kobus ellipsiprymnus		281	1-2	1	240	12000-13000	0	0				Puschmann, 2004
Kobus kob		266	1-2	1	240	4000-5000	0	0				Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Kobus leche			1-2	1	240	5000-10000	0	0			22 18-24	Puschmann, 2004
Litocranius walleri		195-212	1-2	1-2	180	3000	0	0			12 18	Puschmann, 2004
Madoqua kirkii	5300	174	1	1	42	590	0	0			6	Dittrich, 1969
Nemorhaedus goral		215-245	1-2	1	120		0	0			1.5-2.5 y	Puschmann, 2004
Neotragus moschatus		168-177	1-2	1	120-150	860-925	0	0			11-14	Puschmann, 2004
Onotragus megaceros		237	1-2	1	240	4000-6000	0	0			13-19 20	Puschmann, 2004
Oreamnos americanus		147	1-2	1	120	3000	0	0			30	Asdell, 1964
Oreotragus oreotragus		213-228	1-2	1	120-150		0	0			11-14	Puschmann, 2004
Oryx dammah		248	1	1	240	10900	0	0			21.8-27	Dittrich, 1969
Oryx gazella		266	1-2	1	240	9500-14700	0	0			21	Puschmann, 2004
Oryx leucoryx		260-270	1	1	240		0	0			24	Puschmann, 2004
Ourebia ourebia		198-210	1-2	1	120-150	1600-2500	0	0			11-14	Puschmann, 2004
Ovibos moschatus	300000	240	1-2	1	139	8943-9806	0	0			3-5 y	Banks, 1978
Ovis ammon		149-165	1-2	1	120		0	0			1.5-2.5 y	Puschmann, 2004
Ovis aries	30000	150	1-2	1	120	4000	0	0			1.5-2.5 y	Asdell, 1964
Ovis canadensis	61360	180	1	1	120-150	4082	0	0			27	Asdell, 1964
Pseudois nayaur		160	1-2	1	150-180		0	0			18	Puschmann, 2004
Raphiceros campestris		168-177	1-2	1	120-150	900	0	0			11-14	Puschmann, 2004
Rupicapra rupicapra	34000	150-160	1	1	120	3000-5000	0	0			1.5-2 2-3 y	Asdell, 1964
Saiga tatarica	45000	145	1-2	1	60-75	3200	0	0			7-8 19	Asdell, 1964
Sylvicapra grimmia		210	1-2	1	120-150		0	0			11-14	Puschmann,2004
Syncerus caffer	447000	330	1	0.5	180	35570	0	0			1.5-2.5 y	Mentis, 1972
Tragelaphus spekei		251	1-2	1	240	3300-4500	0	0			8-17 10-17	Puschmann, 2004

	Reproduction					Development						
Order /Taxon	Female Adult Weight (g)	Gestation (days)	Range or Mean Litter Size	Aver-age Litters/ Year	Duration of Lactation (days)	Weight of Neonate (g)	Age of Eye Opening (days)	Age of Furring (days)	Attach-ment / First off Teat (days)	Perma-nent Pouch Exit (days)	Age at First Mating (months) ♀ ♂	References
Tetracerus quadricornis		183-240	1-2	1	240		0	0			24	Puschmann, 2004
Tragelaphus angasi		229-245	1-2	1	240	5200-7000	0	0			11-15 16	Puschmann, 2004
Tragelaphus strepsiceros		275	1	1			0	0			14	NZP records
Tragelaphus euryceros		285	1-2	1	240	17000-22300	0	0			20-31 20	Puschmann, 2004
Tragelaphus imberbis		236-264	1-2	1	240	4800-7600	0	0				Puschmann, 2004
Tragelaphus oryx	300000	266	1	1		27400	0	0			3 4 y	NZP records
Tragelaphus scriptus	28600	160	1	1		3450	0	0			18.7	Dittrich, 1969

Acknowledgements

I wish to express my gratitude to all those who have given me valuable assistance during this project. I am very grateful to Prof. Ulrich Zeller for giving me the opportunity to carry out this work. My special thanks to him for the provision of a workplace, his helpful suggestions and the confidence in me and my work. I would also like to thank my co-supervisors Prof. Marilyn B. Renfree and Dr. Barbara Tzschentke for their help, advice and support. My special thanks for their valuable discussions and criticisms of this work.

There are numerous other people who have assisted me in the course of this project. Julia Schultz deserves special mention for her technical assistance, continous support and friendship. The animal keepers of the animal facility of the Institute of Zoology at the Museum of Natural History Berlin, especially Frank Postleb and Anette Billep were very helpful in the care and breeding of the animals and their assistance anytime I needed. I would also like to mention the valuable assistance of Jutta Zeller with the histological processing of the material. Her good temper was supportively during the long days in the lab. I thank also Gabriele Drescher for her assistance with the processing of the electron microscopic material and her readiness to help with technical problems of the TEM and SEM. Also I like to thank Carsten Lüter for his help and debugging aid at the TEM. Hendrik Schneider, the precision mechanic was very innovative and helpful in constructing a respiration chamber for my study.

Dr. Oliver Janke deserves special mention for his technical assistance and continous support with the respiratory measurements and veterinary care. I am also very grateful to him for the reading and comments of this work.

I also have to mention my colleagues who in one way or the other have made my work a pleasure. I would like to mention Drs. Peter Bartsch and Peter Giere who always had a piece of advice at difficult times and were always helpful in proofreading. I thank Swetlana Siniza, Friederike Kachel, Mirco Plath and Jan Ihlau for the work discussions and their company in the lab and during the lunchbreaks.

There are also some people in Australia I would like to thank for their support during my research stay at the Melbourne University. First of all, I thank Prof. M.B. Renfree for her hospitality. The group of Prof. Renfree, especially Sue Osborne, Terry Fletcher and Geoff Shaw, but also all students of the research group were very helpful providing me with tammar wallabies and their assistance anytime I needed. Alan Lambell, the chief technical officer of the Department of Physiology at Melbourne University was very helpful with the metabolic measurements of the tammar wallabies in Australia.

I would also like to thank Prof. Eberhard Fuchs and Dr. Barthel Schmelting from the German

Primate Centre for the lending of tupaias, what made it possible to include this species in the study. Furthermore, I am grateful to Dr. Klaus Rudloff from the Tierpark Berlin-Friedrichsfelde and Prof. Rolf Gattermann from the Martin-Luther-University Halle for the delivery of animals, which was essential to establish breeding colonies for the study.

This work was fascilitated by the German Research Foundation (DFG) as part of the Graduate Research Programm 503 and a grant of the “Berliner Programm zur Förderung der Chancengleichheit von Frauen”.

During the course of this work my family was always there whenever I needed it. I am grateful for their support and love they give to me.

Finally, I would like to thank my husband for his help, advice, support all the time and patience through this undertaking. Monti and Tami, thank you for being there.

Thank you for the support and understanding which you all have shown.Erklärung

Erklärung

Hiermit versichere ich, dass die vorliegende Dissertation

Reproductive strategies of K/T-crossing Theria: Neonate and postnatal development of the morphotype of Marsupialia and Placentalia (Mammalia)

Von mir selbstständig und ausschließlich unter Verwendung der in der Arbeit angegebenen Quellen und aufgeführten Hilfsmittel angefertigt wurde. Die Dissertation wurde in dieser oder einer ähnlichen Form bei keiner anderen Hochschule eingereicht und hat noch keinem sonstigen Prüfungszweck gedient. Ich besitze einen entsprechenden Doktorgrad nicht und habe mich auch nicht anderweitig um einen solchen beworben.

Berlin, 6. Februar 2006